DETERMINANTS OF BONE MINERAL MASS IN ELITE FEMALE ATHLETES

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This thesis is dedicated to my late father

Dr Lionel Wolman.

DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

AUTHOR'S EXPERIENCE

After obtaining MB, ChB from the University of Manchester in 1981, the author gained MRCP(UK) in 1984. From 1984 to 1987, he trained as a registrar in General Medicine and Rheumatology. From 1987 to 1990 he has continued in Rheumatology at the British Olympic Medical Centre while working on this thesis.

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CHAPTER 1: ABSTRACT

In this study, the association between exercise, menstrual status and bone mineral density has been investigated in 67 elite, female athletes. Bone density was measured at 3 sites, the lumbar spine, the proximal femur and the femoral shaft. At the start of the study there were 25 with amenorrhoea, 27 were eumenorrhoeic and 15 were taking the oral contraceptive. The bone density in the lumbar spine and proximal femur was significantly lower in those with amenorrhoea ($P < 0.0001$ and $P = 0.01$ respectively). Bone density at the femoral midshaft (cortical bone) showed no significant difference between the menstrual groups. The eumenorrhoeics and oral contraceptive takers had similar levels of bone density at all 3 sites.

Bone turnover measured biochemically was highest amongst the amenorrhoeics and lowest in the oral contraceptive takers.

The athletes were divided into 3 sporting groups; 36 rowers, 21 runners and 10 ballet dancers. Rowers had significantly higher spinal bone density levels than the other athletes ($P = 0.05$) which partially offset the bone loss incurred by low oestrogen status. Runners had significantly higher bone density levels in the femoral midshaft ($P = 0.0026$).

Spinal bone density and aerobic capacity ($\dot{V}O_2max$) were significantly related. Amongst "normal" menstrual status athletes this relationship was positive and linear (P= 0.043), whereas amongst the amenorrhoeics it was non-linear, with bone density decreasing at higher levels of $\dot{V}O_2max$ (P= 0.015).

The relationship between daily calcium intake and bone density was also studied. There was a strong, positive linear correlation between calcium intake and spinal bone density which applied equally to the low and normal menstrual status athletes (P= 0.002). No relationship was found between calcium intake and proximal femur or femoral shaft bone density.

This thesis has investigated some of the factors that influence bone density levels in amenorrhoeic athletes. More generally it has been concerned with the effect of 3 factors, namely oestrogen status, physical activity and dietary calcium intake, on bone density levels in young adult women. Provided the oestrogen status remains normal, intensive exercise will enhance bone mass leading to increases of between 10-20% compared to eumenorrhoeic, sedentary women. These impressive increases in bone density may reduce the risk of osteoporosis in later life.

The results support the hypothesis that exercise regimes applied to young female adults and to women in the peri- and post-menopausal period may provide useful additional strategies

in the prevention and management of postmenopausal osteoporosis. These concepts should form the basis for future research.

CHAPTER 2: BACKGROUND

2.1 ATHLETIC AMENORRHOEA

Since the late 1970's amenorrhoea has become a well recognised feature of endurance training (Dale et al, 1979). Before then there were very few endurance events for women and so intense aerobic training and its associated hormonal changes were unusual. Since then the incidence of amenorrhoea has increased as rigorous training routines have been employed by more female athletes.

The incidence of athletic amenorrhoea varies between different sports and strongly depends on the intensity of training. In a study of athletes entering national collegiate championships in America (Feicht Sanborn et al, 1982) the incidence amongst runners was 25.7%, swimmers 12.3% and cyclists 12.1%. In another study the incidence amongst ballet dancers was 55% (Frisch et al, 1980). Very little work has been done in other sports . The cause of athletic amenorrhoea is unknown but it is associated with the following factors outlined in sections 2.1.1 - 2.1.6.

2.1.1. NUTRITIONAL STATUS

Calorie restriction is an important factor in the development of amenorrhoea, the most extreme example being anorexia nervosa (Walsh et al, 1980). Calorie restriction is important in certain sports that require a high power-to-weight ratio such as ballet, cycling, gymnastics and long distance running. It is also important in sports where there are weight restrictions. This applies to Lightweight (LW) rowers who must be at or below 59 Kg to compete whereas Heavyweights (HW) can be any weight.

Previous research has shown that the calorie intake of amenorrhoeic athletes is significantly lower than that of their eumenorrhoeic counterparts even when matched for training intensity (Drinkwater et al, 1984; Nelson, Fisher et al, 1986). This would suggest an increased efficiency of nutrient utilization and a reduced energy expenditure during non-training activities such as sleep. Studies have also shown other nutritional differences between amenorrhoeic and eumenorrhoeic athletes (Deuster et al, 1986; Lloyd, Buchanan et al, 1987) but it is uncertain whether these represent the cause or effect of amenorrhoea.

2.1.2. TRAINING INTENSITY

The incidence of amenorrhoea amongst athletes is related to the intensity of training (Feicht et al, 1978; Feicht Sanborn et al, 1982). In athletes running 60-80 miles/week the incidence of menstrual abnormalities is between 50-60% whereas in those running 20 miles/week it is only 20% (Feicht et al, 1978).

2.1.3. BODY COMPOSITION

Increased training intensity and calorie restriction leads to weight loss and reduction in body fat which may be important in the pathophysiology of amenorrhoea. Previous studies have shown an association between amenorrhoea and low body weight (Speroff and Redwine, 1980) and low levels of body fat (Schwartz et al, 1981; Glass et al, 1987). Frisch and McArthur (1974) proposed that a critical percentage of body fat is required both for the onset of menarche (17%) and for maintenance of eumenorrhoea (22%). A possible explanation for this is the conversion of androgens to oestrogens, in particular oestrone, that occurs in peripheral fat cells (Nimrod and Ryan, 1975). In lean women with reduced levels of body fat, peripheral conversion of androgens to oestrogens is impaired leading to altered metabolism of oestradiol. This relative fall in oestrogenic activity may disrupt menstruation.

In other studies no differences in body fat between the menstrual groups have been found (Sanborn et al, 1987). This discrepancy can possibly be explained first by the differing selection criteria used in each of the studies and second by the well recognised lack of agreement between the different methods of measuring body fat (Cumming and Rebar, 1984).

2.1.4. PREVIOUS MENSTRUAL IRREGULARITY

Amenorrhoeic athletes frequently give a past history of menstrual irregularity (Schwartz et al, 1981), a late menarche (Feicht et al, 1978) and are more likely to be nulliparous than their eumenorrhoeic counterparts (Dale et al, 1979). This suggests that exercise may increase the risk in those individuals who are already susceptible to menstrual irregularity rather than being the cause per se.

2.1.5. AGE

Menstrual dysfunction is more common in younger athletes (Speroff and Redwine, 1980). Furthermore there is evidence to show that those who take up serious training well before puberty not only delay the menarche but also are more at risk of menstrual irregularity later (Frisch, Gotz-Webergen et al, 1981; Wakat et al, 1982). In the perimenarchal period major changes take place

in the hypothalamic-pituitary-ovarian axis. Extreme exercise around this time may affect these changes and might help to account for the features of menstrual dysfunction.

2.1.6. STRESS

Emotional stress can be associated with menstrual dysfunction and this is thought by some to be important in the pathogenesis of amenorrhoea especially in anorexia nervosa. Gadpaille et al (1987) recognised similarities between amenorrhoeic athletes and patients with anorexia nervosa, in particular eating disorders, major affective disorders and compulsive behaviour. These features were not present amongst the eumenorrhoeic athletic controls. Schwartz et al (1981) found that amenorrhoeic runners considered their training more stressful than did eumenorrhoeic runners.

All these factors may have an effect on the hypothalamic-pituitary-ovarian axis which is disrupted in the development of athletic amenorrhoea. There is evidence of loss of the pulsatile release of gonadotrophin releasing hormone (GnRH) from the hypothalamus in exercise (Veldhuis et al, 1985; Cumming, Vickivic et al, 1985). This, in turn, leads to a reduction in pulsatile release of luteinizing hormone (LH) from the pituitary which will then affect ovarian function. Dopamine, noradrenaline and endorphin are all thought to play a role in the release of GnRH

and changes in these hormones during exercise may be responsible for the development of amenorrhoea (Noakes and Van Gend, 1988). Changes in levels of melatonin and serotonin also occur during exercise and may also affect the menstrual cycle (McCann et al, 1984).

2.2 FACTORS AFFECTING BONE MINERAL DENSITY (BMD).

2.2.1 AMENORRHOEA AND HYPO- OESTROGENAEMIA.

Amenorrhoea and oligomenorrhoea are usually associated with significant falls in oestrogen. The importance of ovarian function, in particular oestrogen, in maintaining skeletal integrity was first appreciated by Albright et al in 1941 when they suggested a link between the menopause and osteoporosis. Aitken et al (1973) provided further evidence of this when they investigated the effects of oophorectomy for non-malignant disease in 258 pre-menopausal women and showed a significant increase in the prevalence of osteoporosis within 3-6 years of the operation.

With the advent of the technology for measuring BMD accurately, recent studies have shown that there is a rapid fall in BMD around the time of the menopause and that this may persist for up to 8 years (Riggs and Melton, 1986). This fall is around 5% per year compared to about 0.4% per year in premenopausal women.

Amenorrhoea and hypo-oestrogenaemia occur in certain circumstances in premenopausal women and changes in BMD in relation to this have been investigated.

2.2.1.1 ANOREXIA NERVOSA (AN)

Amenorrhoea is very common in this disorder. Rigotti et al (1984) showed that the BMD in the radius of the non-dominant arm of 18 amenorrhoeic AN patients was significantly lower than in 28 eumenorrhoeic controls. Furthermore 2 of the patients with AN developed vertebral crush fractures and biopsy in one of these revealed osteoporosis. Those AN patients with high levels of physical activity had greater BMD values than those who were less active. Other studies (Szmukler et al, 1985; Treasure et al, 1987) have shown similar effects on BMD.

2.2.1.2 HYPERPROLACTINAEMIA

Amenorrhoea occurs in hyperprolactinaemia because the high serum levels of prolactin disrupt normal hypothalamic-pituitary-ovarian axis function. Klibanski et al (1980) measured BMD in the distal radius of 14 patients with hyperprolactinaemic amenorrhoea and compared this with 16 age-matched controls. BMD was significantly lower in the patient group and furthermore the patients with the highest serum oestradiol concentrations (i.e. above 20 pg/ml) had greater BMD values than those with concentrations below this level. Cann, Martin et al, (1984) showed decreased vertebral BMD in 9 patients with this condition compared to a eumenorrhoeic control group.

2.2.1.3 LUTEINIZING HORMONE RELEASING HORMONE (LHRH) TREATMENT

Buserelin, an LHRH agonist, causes a reversable suppression of gonadotrophin secretion leading to hypo-oestrogenaemia and amenorrhoea. It is used in the treatment of endometriosis. Matta et al (1987) studied 13 patients with this disease. After 6 months treatment with buserelin there was a significant fall in trabecular BMD in the lumbar spine.

2.2.1.4 ATHLETIC AMENORRHOEA

In 1984 3 independent studies showed that amenorrhoeic athletes have reduced levels of BMD (Cann, Martin et al; Drinkwater et al; Lindberg, Fears et al) - see chapter 2.3.

2.2.1.5 THE EFFECT OF THE RETURN OF MENSTRUATION

Treasure et al (1987) showed that BMD was significantly lower in patients with active Anorexia Nervosa compared to those who had recovered from the disorder. However this study was unable to separate the effects of weight gain and eumenorrhoea on BMD in the recovered group. Drinkwater, Nilson, Ott et al (1986) and Lindberg, Powell et al (1987) investigated this effect in amenorrhoeic athletes. Both studies showed an increase in BMD in association with the return of eumenorrhoea. Matta et al (1987) showed that 6 months of amenorrhoea induced by buserelin produced

a fall in the trabecular BMD (see above). When these patients were studied 6 months after cessation of treatment (Matta et al, 1988) menstruation had returned to normal and the BMD had nearly returned to pretreatment levels.

There is therefore clear evidence showing falls in BMD in pre-menopausal women who have episodes of amenorrhoea. However there is little information on the rate at which BMD is lost and whether this varies according to the duration of amenorrhoea.

2.2.2 PHYSICAL ACTIVITY.

As long ago as 1892, Wolff recognised that bone tissue adapts to the functional forces acting upon it. In the last 40 years there has been accumulating data on the association between physical activity and BMD.

Human studies can be divided into those reporting the effects of either decreased or increased exercise levels on bone metabolism.

2.2.2.1 CHANGES IN BONE DURING IMMOBILIZATION AND WEIGHTLESSNESS.

Deitrick et al (1948) found that during a period of bed rest, healthy men showed an increased excretion of calcium but no radiologically visible osteoporosis. Young subjects bed rested for periods of up to 36 weeks, negative calcium balance in the order of 200-300 mg per day occurs together with increased urinary hydroxyproline excretion (Lockwood et al, 1973). BMD in the calcaneus decreased by up to 30% in this study. During immobilisation BMD, measured by dual photon absorptiometry, falls by 1-2% per week at sites of trabecular bone and by 1% per month at cortical bone sites (Mazess and Whedon, 1983). Andersson and Nilsson (1979) showed an 18% drop in bone density in the proximal tibia in patients operated on for knee ligament injuries and this had not fully recovered one year later despite regaining full mobility.

In those who are exposed to a gravity-free environment, similar effects have been observed. Demineralisation of bone was demonstrated by Mack et al during the Gemini-Titan orbital flights (1967) and during the Apollo project (1971). Those astronauts who participated in a programme of isometric and isotonic exercises had smaller loses of bone mineral (1967). Work done on the skylab astronauts (Whedon et al, 1976) showed falls in calcium balance similar to the levels in bedrested subjects.

Bone losses persist for at least 6-9 months. Very few of the studies have gone on beyond 9 months but there is some evidence that these losses slow down after this time (Mazess and Whedon, 1983). Although the bone losses seem to be reversible the period of recovery is several times longer than the period of loss (Mazess and Whedon, 1983). Furthermore no data are available to permit estimation of a magnitude of loss that would represent a "point of no return".

2.2.2.2 CHANGES IN ATHLETES.

Nilsson and Westlin (1971) showed that athletes had significantly higher BMD's in the distal femur than age-matched controls. Amongst the athletes weight-lifters had the highest and swimmers had the lowest BMD's with runners lying in between. Since then several studies have investigated the effect of different types of sport on BMD.

Studies on runners have shown a beneficial effect on BMD. The usual fall in total body calcium with age does not occur in runners (Aloia, Cohn, Ostuni et al, 1978). Williams, Wagner et al (1984) showed that BMD in the os calcis increased in a group of runners (defined as running more than 16 Km per month) compared to age-matched sedentary controls over a 9 month period. Lane et al (1986) showed that BMD in the first lumbar vertebrae was about 40% higher in runners than in matched controls.

Studies on tennis players have shown major differences in BMD between the playing and non-playing arms. In non-athletes BMD in the radius is 6-8% higher in the dominant arm (Karjalainen and Alhava, 1976). In active male tennis players BMD is 13% higher in the radius of the playing arm (Huddleston et al, 1980). However in top class tennis players humeral BMD is up to 40% (Jones et al, 1977; Dalen et al, 1985) and radial BMD 34% higher in the playing arm (Pirnay et al, 1987).

There is conflicting evidence regarding swimming. Nilsson and Westlin (1971) showed that swimmers had greater femoral BMD than non-athletes, but not as great as weight lifters, runners and soccer players. Orwoll et al (1987) showed that male swimmers over the age of 40 years, have significantly greater vertebral BMD's than age-matched controls. However in postmenopausal women there was no difference between the exercising and control groups. Jacobson et al (1984) showed that the BMD in the lumbar spine was similar in female swimmers and age-matched, sedentary controls but was significantly higher in tennis players.

Snyder et al (1986) presented data suggesting that rowing may prevent the development of osteoporosis in the lumbar spine (see chapter 2.3)

2.2.2.3 RELATIONSHIP WITH ACTIVITY LEVEL.

Aloia, Vaswani et al (1988) measured the level of physical activity in 24 healthy, white premenopausal women using a motion sensor. In this cross-sectional study there were significant correlations between physical activity and BMD measured in the spine and total body calcium measured by neutron activation analysis.

At least 3 studies have shown a correlation between BMD and the maximum oxygen uptake ($\dot{V}O_2$ max), a measure of aerobic fitness. Suominen et al (1984) measured BMD in the calcaneus of men between the ages of 31 and 75 and showed a significant correlation with $\dot{V}O_2$max. Chow, Harrison, Brown et al (1986) showed a significant correlation between $\dot{V}O_2$max and total body calcium, measured by neutron activation analysis, in 31 postmenopausal women. Pocock et al (1986) also showed a significant correlation in 38 pre- and 46 postmenopausal women between $\dot{V}O_2$max and BMD measured at the femoral neck and in the lumbar spine.

Other studies have also demonstrated a relationship between muscle mass/strength and BMD. Sinaki et al (1986) showed a close correlation between BMD of the lumbar spine and isometric strength of the back extensors. However both these parameters were positively correlated with height and negatively correlated with age making it difficult to determine an independent effect.

Pogrund et al (1986) showed a weak relationship between psoas width and the development of osteoporosis in the lumbar spine as measured by plain radiograph.

2.2.2.4 EFFECT OF EXERCISE INTERVENTION.

A number of studies have investigated the effect of exercise intervention on age-related bone loss. Aloia, Cohn, Ostuni et al (1978) studied 18 postmenopausal women over a one year period, half of whom exercised for 1 hour, 3 times a week. Those who exercised increased their total body calcium significantly while the level in the non-exercisers decreased. Smith, Reddan et al (1981) followed a group of elderly ladies (average age of 81) for 3 years and showed that an exercising group increased the BMD in the distal radius compared to a control group in whom the BMD decreased. Krolner et al (1982) demonstrated an increase in the BMD of the lumbar spine in a group of exercising postmenopausal women (age range 50-73 yrs) which did not occur in the control group. Chow, Harrison and Notarius (1987) followed up a group of 48 postmenopausal women (age range 50-62) for one year. The subjects were randomised into control and exercise groups and bone mass, determined by neutron activation analysis, was measured at the beginning and end of the study. The exercising groups showed a significant increase in bone mass and in aerobic fitness compared to the control group.

2.2.2.5 ANIMAL STUDIES.

Animal studies have shed further light on the effect of load bearing on bone. Lanyon has completed a series of experiments, measuring strain patterns induced in the avian ulna when external loads were applied. Lanyon and Rubin (1984) showed that bone remodelling is sensitive to dynamic and not static loads. Rubin and Lanyon (1985) showed a direct relationship between the peak strain magnitude and bone remodelling activity. At very low peak strain levels bone loss occured but with increasing strain levels increased bone mass resulted. In work on roosters Rubin and Lanyon (1984) showed that the frequency at which the load is applied to the bone will also exert an effect, with higher frequencies exerting a greater effect. However the osteogenic stimulus was saturated at loading cycles of as low as 36 per day with no additional benefit at higher frequencies. O'Conner et al (1982) revealed, in studies on sheep, that the osteogenic stimulus is also affected by the rate at which the strain changes, with the higher rates producing the greatest effects. Rubin and Lanyon (1985) have also shown that strain distribution is also important, such that even when strain magnitude and strain rate are constant, alteration in the strain distribution will exert an osteogenic effect.

In humans the functional strains induced on bone by weight bearing activity would seem to provide an osteogenic stimulus (see above). From the animal experiments this stimulus could

possibly be enhanced more by performing short periods of intense weight bearing activity rather than by longer periods of repetitive loading which involve lower peak loads (Lanyon, 1989).

2.2.2.6 CONCLUSION

Research on immobilization and weightlessness indicates the importance of stress loading in maintaining skeletal mineralization. Weight-bearing activity is especially important in providing the appropriate stress. Work on tennis players has demonstrated that exercise produces a local effect on the bone and not a general benefit to the whole skeleton. Therefore weight-bearing activity such as walking and running is likely to be more effective in maintaining integrity of the neck of femur and the spine than nonweight-bearing activity such as swimming and cycling. To increase BMD in the wrist and thus reduce the risk of Colles fracture, exercise directed to the arm would be appropriate. However the specific relationship between bone mineral density and the type, the intensity and the frequency of exercise has not been delineated.

2.2.3 CALCIUM.

Over 99% of body calcium is in the skeleton and it is, by far, the most important mineral in bone. Intestinal calcium absorption diminishes with age in both sexes (Bauwens et al, 1986) leading to the rationale for using calcium in the treatment of osteoporosis. However there is only limited evidence showing any benefit from its use in the prevention of osteoporosis.

2.2.3.1 CALCIUM BALANCE STUDIES

Nordin, Horsman, Marshall et al (1979) estimated the daily calcium requirement from 212 calcium balance studies on 84 normal subjects and showed that an intake of about 900 mg is required to ensure that 95% of adults are in balance. Heaney, Recker et al (1978) performed calcium balance studies on 168 perimenopausal women and plotted calcium intake against calcium balance. By performing regression analysis it was shown that to achieve calcium balance, daily intake had to be at least 1000 mg for premenopausal and oestrogen-treated postmenopausal women and 1500 mg for untreated postmenopausal women. This suggested an oestrogen-related shift in calcium requirement across the menopause.

2.2.3.2 CALCIUM INTAKE AND BONE MINERAL DENSITY

Angus et al (1988) showed no correlation between BMD and current calcium intake in 159 women (age range 23-75 years). Riggs, Wahner, Melton et al (1987) assessed calcium intake and measured the rate of change in BMD over a 2-7 year period in 106 women (age range 23-84 years), but were unable to show any correlation between these 2 variables. Kanders et al (1988) investigated 60 young, eumenorrhoeic women (age range 25-34 years) and showed a positive correlation between BMD and calcium intakes up to but not above 800-1000 mg/day, suggesting a threshold effect of calcium intake on BMD.

A series of studies have been published investigating the effect of calcium supplementation on BMD. Horsman et al (1977) and Recker et al (1977) each explored the effect of calcium supplementation in relation to oestrogen treatment in a group of postmenopausal women over a 2 year period. Both studies showed that the BMD fell least in the oestrogen-treated group and most in the "no treatment" control group. The fall in the calcium-treated group lay between these extremes. In a similar study, Riis et al (1987) showed that calcium supplementation had only a minor effect on the loss of cortical bone and no effect on trabecular bone. This was not as effective as oestrogen therapy. Both Lamke et al (1978) and Hansson and Roos (1987) showed that calcium supplementation alone had no effect on bone loss in a group of postmenopausal women.

2.2.3.3 CALCIUM INTAKE AND FRACTURE RISK

Matkovic et al (1979) showed that in a district of Yugoslavia where there was a high calcium intake in the form of dairy products, the frequency of hip fracture was 50% below that in a nearby community with a low intake. However the energy intake was also higher in the high-intake calcium community even though body weight was similar in the two communities, suggesting that physical activity levels were higher in the high intake group and that this could be responsible for the reduced fracture rate. Holbrook et al (1988) measured the calcium intake in 957 men and women aged 50 to 79 years. At follow up 12 to 14 years later the risk of hip fracture was shown to be inversely associated with calcium intake. This association persisted even after adjustment for smoking, exercise and obesity. Studies by Nordin, Horsman, Crilly et al (1980) and by Riggs, Seeman et al (1982) suggested that calcium supplementation could decrease the risk of vertebral crush fracture in patients with spinal osteoporosis.

2.2.3.4 CALCIUM REQUIREMENTS

The recommended daily intake (RDI) for a nutrient can be defined as the amount that is sufficient or more than sufficient for the nutritional needs of practically all healthy persons in a population (HMSO, 1969). In the UK the RDI for calcium is 500 mg for adults, rising to 1200 mg in pregnancy and lactation. The RDI has been unchanged in the UK for over 20 years. In 1980 in the

USA the RDI was increased to 800 mg. The 1984 US Consensus Development Conference recommended that calcium intake should be increased to 1000-1500 mg/day based on the work of Heaney, Recker et al (1978)- see above. The 1987 international Symposium on Osteoporosis in Denmark recommended a calcium intake of around 800 mg for caucasian women with a higher requirement during linear growth, pregnancy and lactation.

Obligatory faecal and urinary losses amount to about 150-250 mg per day (Heaney, Gallagher et al, 1982). Calcium uptake by the bone varies and is at a peak during the first months of life and again during the adolescent growth spurt when up to 400 mg daily may be retained (Kanis and Passmore, 1989). At other times retention of calcium may be as low as 20 mg daily. Normal adults adapt to decreased calcium intake mainly by increasing the fraction of dietary calcium absorbed but the ability to adapt is impaired by ageing. Vitamin D is a major regulator of intestinal calcium absorption and so may play an important role in calcium balance (see chapter 2.4.1.1).

2.2.3.5 CONCLUSION

Calcium requirements vary during the life cycle and are increased during pregnancy, lactation and the growth spurts. High calcium intake may be of benefit after the menopause when it may marginally increase BMD and reduce fracture risk. High intake may also be of some benefit in young adults in achieving a high peak

bone mass. Calcium may act as a "threshold" nutrient where benefit is obtained up to a certain level of intake but not beyond that point. However there is still some disagreement about the RDI for calcium in different age groups and further studies in this area are needed.

2.3 ATHLETIC AMENORRHOEA AND OSTEOPOROSIS.

Amenorrhoea in endurance athletes was first recognised in the late 1970's (chapter 2.1). It was thought initially that the high level of exercise would protect such women from developing osteoporosis. However although Gonzalez (1982) suggested that this might not be the case, it was not until 1984 that there was clear evidence of osteoporosis in amenorrhoeic athletes.

2.3.1 INITIAL REPORTS

Cann, Martin et al (1984) investigated 10 amenorrhoeic athletes and compared them to 25 women with amenorrhoea for other reasons and with 50 eumenorrhoeic, sedentary controls. All the groups studied were of similar age. BMD in the lumbar spine (measured by CT scanning) was significantly lower in the amenorrhoeic groups than in the controls but there was no difference in BMD at the radius (measured by single photon absorptiometry). There was no difference in BMD between the amenorrhoeic groups. However as this study was not designed to investigate BMD changes in athletic amenorrhoea, there are no data regarding training intensity and there was even some doubt as to whether exercise was in fact the cause of amenorrhoea.

Drinkwater et al (1984) measured BMD in the lumbar spine (using dual photon absorptiometry) and in the radius (using single photon absorptiometry) in 14 amenorrhoeic and 14 eumenorrhoeic runners matched for age, height and weight. The training intensity was recorded with the amenorrhoeics running 42 miles/week and the eumenorrhoeics 25 miles/week. The nutritional intake was similar in the 2 groups. BMD in the lumbar spine was significantly lower in the amenorrhoeic group but there was no difference in radial measurements between the 2 groups.

Lindberg, Fears et al (1984) investigated 11 amenorrhoeic runners and showed decreased levels of BMD in both the lumbar spine and the radius when compared to eumenorrhoeic runners and to eumenorrhoeic, sedentary controls.

Marcus et al (1985) studied 17 elite female distance runners, 11 of whom had amenorrhoea and the remainder were eumenorrhoeic. The amenorrhoeic athletes had significantly lower BMD in the lumbar spine than the eumenorrhoeic athletes but greater levels than a group of amenorrhoeic non-athletes, suggesting that extreme weight-bearing exercise partially overcomes the adverse skeletal effects of oestrogen deprivation.

2.3.2 STUDIES IN OTHER SPORTS

Most of the studies have been restricted to runners. However Snyder et al (1986) investigated 16 elite lightweight rowers, 7 being eumenorrhoeic, 5 oligomenorrhoeic and 4 amenorrhoeic. BMD in the lumbar spine (measured by dual photon absorptiometry) showed no difference between the 3 groups and was similar to a group of non-athletic, eumenorrhoeic controls. It was suggested that the extreme trunk exercise in this sport might protect the spine from the effects of low oestrogen status.

2.3.3 NUTRITIONAL AND METABOLIC STUDIES

Nutritional abnormalities have been investigated in amenorrhoeic athletes. Drinkwater et al (1984) and Nelson, Fisher et al (1986) have both shown no difference in calcium intake between amenorrhoeic and eumenorrhoeic athletes. They also showed that amenorrhoeic athletes have a lower daily energy intake. Lloyd, Buchanan et al (1987) showed that dietary intake of fibre was significantly higher in a group of oligomenorrhoeic athletes compared to the eumenorrhoeic group. This high intake may alter levels of circulating oestrogen and may also decrease calcium absorption from the gut, the mechanisms of which are uncertain.

Marcus et al (1985) investigated metabolic features in a group of amenorrhoeic and eumenorrhoeic runners. Serum triiodothyronine was lower amongst the amenorrhoeic athletes, suggesting a lower metabolic rate which was possibly a reflection of the low calorie intake seen in this group. Serum levels of calcium and phosphate were similar in the 2 groups. Urinary calcium excretion and serum alkaline phosphatase levels were higher in the amenorrhoeic group, which although they did not reach the level of significance, may have represented a higher bone turnover state. Serum immunoreactive parathyroid hormone (PTH) and 25-hydroxyvitamin D were slightly lower in the amenorrhoeic group but again this did not reach the level of significance. However serum calcitriol was significantly lower in the amenorrhoeic group. This study involved only small numbers which may account for the inability to show a significant difference in PTH between the 2 groups. Cook et al (1987) measured c-terminal midmolecule PTH in 19 oligomenorrhoeic and 17 eumenorrhoeic athletes and showed significantly lower levels in the oligomenorrhoeic group. This was thought to be due to its interaction with oestrogen (see chapter 2.4.1.4).

2.3.4 FRACTURE RISK

Amongst the 14 amenorrhoeic athletes in Drinkwater's study (1984) 2 had vertebral BMD's below the fracture threshold, as defined by Riggs, Wahner et al (1981), suggesting an increased risk of compression fracture. Both Lindberg, Fears et al (1984) and

Marcus et al (1985) showed a statistically higher incidence of cortical stress fractures amongst amenorrhoeic runners than in their eumenorrhoeic counterparts but the numbers involved were small. Lloyd, Triantafyllou et al (1986), in a much larger questionnaire-based study, also showed that the incidence of fractures was higher amongst amenorrhoeic athletes. However in this study the nature and site of the fracture was not specified and the validity of the results has been questioned (Walker, 1987). Furthermore only in the study by Marcus is there any attempt to control for training intensity. This is relevant as not only will it influence the incidence of stress fractures but also the incidence of menstrual irregularity (see above). As stress fractures occur at sites of cortical bone and as most of the studies on athletic amenorrhoea have shown changes only in trabecular bone, it is still unclear whether amenorrhoea is the cause of such fractures (figure 2.3.4).

2.3.5 LONGITUDINAL STUDIES

None of the above studies have investigated the effects of prolonged amenorrhoea or of the return of normal menstruation on BMD. Parker Jones et al (1985) investigated 39 women with amenorrhoea, including 8 athletes, and showed a significant negative correlation between BMD in the radius and months of amenorrhoea.

Drinkwater et al (1986) re-evaluated the subjects from their original study in 1984. The time gap was 15 months. Nine of the original 14 amenorrhoeic athletes were retested together with 7 of the 14 eumenorrhoeics (see above). Seven of the 9 had regained their menstrual cycles and their lumbar spine BMD had risen by 6.3%. The BMD in the 2 who had remained amenorrhoeic fell by a further 3.4%. The BMD in the group who remained eumenorrhoeic remained unchanged.

Lindberg, Powell et al (1987) re-evaluated 7 of the original 11 amenorrhoeic athletes from 1984 (see above). Again the time gap was 15 months. In the 4 who had regained menstruation BMD in the lumbar spine rose by 6.7% whereas it remained unchanged in the 3 athletes who continued to be amenorrhoeic. In the group who had regained menstruation there had been an associated reduction in weekly running distance and an increase in body weight.

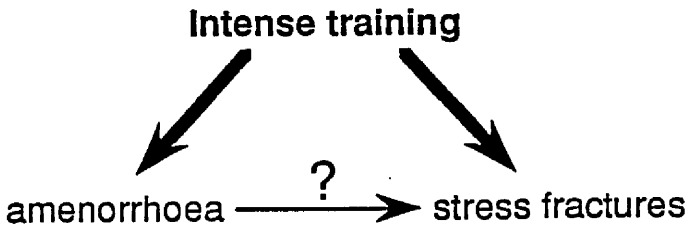

FIGURE 2.3.4:

The Relationship between Intense Training, Amenorrhoea and Stress Fractures.

2.4 BIOCHEMICAL CHANGES IN POSTMENOPAUSAL OSTEOPOROSIS.

During the development of osteoporosis there are associated biochemical changes. These can be divided into those which involve changes that may be important in the pathogenesis and those which reflect the effect of the disorder, in particular changes in bone turnover. In order to understand these changes it is important to appreciate the factors that control normal bone metabolism.

2.4.1 CALCIUM HOMEOSTASIS AND CONTROL OF BONE METABOLISM

The skeleton is composed of two types of bone, cortical and trabecular. Approximately 80% is cortical and 20% trabecular. The relative amounts of these varies at different sites in the skeleton. For example the vertebrae consist predominantly of trabecular bone (Nottestad et al, 1987) whereas over 90% of the midshaft of a long bone consists of cortical bone (Bohr and Schaadt, 1985). In the hip there are roughly equal amounts of each. Trabecular bone has turnover rate several times that of cortical bone and is more sensitive to the effects of changing oestrogen levels.

The organic matrix of bone is 90% collagen (Smith R, 1987). The remaining 10% consists of a variety of non-collagenous substances including osteocalcin, also called Bone Gla-containing Protein

(BGP)- see below. Mineral, in the form of a calcium phosphate complex, is deposited on the collagenous matrix to which it adds structural rigidity. Although 99% of the body's calcium is present in bone (chapter 2.2.3), the remainder has many other important functions including involvement in normal muscle physiology. The main hormones known to affect extracellular calcium homeostasis are parathyroid hormone (PTH), vitamin D and calcitonin. Other hormones, including the sex hormones and the somatomedins, also play a significant role but this is less clearly understood. Plasma calcium is closely regulated between about 2.25 mmol/l and 2.60 mmol/l and is either ionized, protein-bound or complexed. It is the ionized form which is important in the control of PTH and calcitonin secretion. The control of plasma phosphate depends on the glomerular filtration rate and on renal tubular reabsorption.

2.4.1.1 Vitamin D

There are 2 forms of vitamin D. One, vitamin D_3, cholecalciferol, is synthesised in the skin by the action of ultraviolet light on the precursor 7-dehydrocholesterol. It is also present in the diet especially in dairy products and oily fishes. The other, vitamin D_2, ergocalciferol, is synthetic but is still present in the diet especially in some margarines.

Both D_2 and D_3 are transported to the liver where they undergo a 25-hydroxylation in the hepatic microsomes. This 25-hydroxylated derivative circulates in the plasma and is a good index of vitamin D status. Further hydroxylation occurs in the kidney to either 1,25 dihydroxy-vitamin D or 24,25 dihydroxy-vitamin D. $1,25(OH)_2D$ is synthesized under the influence of 1-alpha-hydroxylase which is stimulated by low plasma calcium, low plasma phosphate and by PTH. Production is controlled by a negative feedback mechanism.

$1,25(OH)_2D$ increases plasma calcium by stimulating bone resorption and by increasing calcium absorption across the gut, in part by controlling the synthesis of a calcium-binding protein within the small intestinal cell. $24,25(OH)_2D$ is less active and its formation is favoured by normal plasma calcium levels.

2.4.1.2 Parathyroid Hormone

This is an 84 amino acid hormone which is synthesised in the parathyroid gland. Specific cleavage of PTH occurs in the liver leading to the main circulating fragments. Assays can measure either -C terminal,-N terminal or the middle molecule. The -C terminal fragment is biologically inactive and has a long half-life; the -N terminal fragment is biologically active but has a shorter half-life.

Secretion of PTH is stimulated by hypocalcaemia and this tends to restore plasma calcium to normal. Plasma levels of PTH tend to rise with age. PTH causes an increase in renal reabsorption and bone resorption of calcium. Increased intestinal absorption of calcium also occurs which, in part is due to stimulating the renal 1-alpha-hydroxylase enzyme leading to increased production of $1,25(OH)_2D$ (Silverberg et al, 1989). $1,25(OH)_2D$ in turn depresses production of mRNA for PTH completing the negative feedback loop.

PTH also has an independent effect in decreasing renal tubular reabsorption of phosphate.

2.4.1.3 Calcitonin

This 32 residue polypeptide is secreted by the C cells of the thyroid gland. Its main effect on bone is to reduce osteoclastic bone resorption. Because of this effect, exogenous calcitonin is used in the treatment of Pagets disease.

Calcitonin levels are much lower in females than in males (Heath and Sizemore, 1977) and it has been suggested that this may be related to the difference in prevalence of osteoporosis. Calcitonin secretion also appears to decline with age (Deftos, Weisman et al, 1980) although not all studies have confirmed this

(Stevenson, 1988). Some studies have shown no difference in calcitonin between postmenopausal women with and without osteoporosis.

2.4.1.4 Oestrogen

The exact mechanism of action of oestrogens on bone has still not been established. Until recently, studies had been unable to show the presence of oestrogen receptors in bone leading to speculation about possible indirect actions. However recent data are suggestive of the presence of oestrogen receptors in low concentrations in human osteoblasts (Erikson et al, 1987; Komm et al, 1987).

One possible hypothesis (Riggs, 1981) is that the loss of ovarian function leads directly to bone resorption, by a mechanism which is uncertain. The plasma calcium would then tend to rise causing a secondary suppression of PTH and $1,25(OH)_2D$. These do occur at the menopause and both changes are reversed with oestrogen replacement (Stevenson, Abeyasekera et al, 1983).

Several mechanisms have been suggested for the indirect action of oestrogens on bone. In postmenopausal women, oestrogen therapy leads to inhibition of bone resorption with a delayed effect on bone formation and it has been suggested that it acts by decreasing the sensitivity of bone to PTH.

Stevenson, Abeyasekera et al (1981) demonstrated that the administration of oestrogen raises the plasma immunoreactive calcitonin level, suggesting that this may act as the mediator of oestrogen therapy. However, other studies have failed to show this response (Tiegs et al, 1985) leading to controversy concerning the importance of calcitonin in the pathogenesis of osteoporosis.

Whether the changes in PTH, vitamin D and calcitonin are the mediators of the action of oestrogen or whether they represent the response to changes in plasma calcium caused by the direct action of oestrogen remains unclear.

2.4.1.5 Other Hormones

The growth and composition of the skeleton is affected by other hormones. Growth hormone stimulates the production of cartilage and bone with deficiency leading to osteoporosis (Smith R, 1987). Androgens may also stimulate the production of bone. Hypogonadism in the male is associated with osteoporosis. Thyroid hormones stimulate bone turnover. In thyrotoxicosis bone resorption exceeds formation and this is associated with the development of osteoporosis (Smith R, 1987). The importance of these hormones in maintaining normal bone metabolism and the way they may interact with each other is not fully understood.

2.4.2 BIOCHEMICAL MARKERS OF BONE TURNOVER

2.4.2.1 Bone Formation

Serum alkaline phosphatase (ALP) and serum osteocalcin (BGP) are both used as markers for bone formation.

Alkaline Phosphatase

ALP is released from the osteoblast into the circulation but it is also released from cells in the liver and intestine (Taylor et al, 1987). Total serum ALP in normal adult subjects contains approximately equal parts of two isoenzymes: one derived from liver and the other derived from bone. It is therefore a non-specific marker of bone formation. Skeletal ALP isoenzyme is a more specific marker but there have been few studies evaluating its use in metabolic bone disease. Stepan et al (1987) however have shown a significant positive correlation between skeletal ALP isoenzyme and BGP in oophorectomy and primary hyperparathyroidism patients.

Osteocalcin

This is also referred to as bone Gla protein (BGP). It is the most abundant non-collagenous protein found in bone and is synthesised by osteoblasts (Taylor et al, 1987). Serum BGP

reflects the rate of bone formation as assessed by histomorphometric methods. In some studies serum levels of BGP correlated with serum ALP (Epstein et al, 1984; Podenphant et al, 1985; Stepan et al, 1987), while others (Gundberg et al, 1983; Yasumura et al, 1987) were unable to show a relationship between these 2 parameters.

BGP is a small protein (6000 daltons) and contains 49 amino acids. It contains 3 residues of glutamic acid which are carboxylated to carboxyglutamic acid by a process which is vitamin K dependent. The carboxylated (GLA) form binds strongly to hydroxyapatite and is therefore thought to be the active form of the protein (Menon et al, 1987). The uncarboxylated (GLU) form is thought to be inactive. Warfarin, a vitamin K antagonist, inhibits the conversion of GLU to GLA (Price et al, 1981) and increases the ratio of inactive to active forms of osteocalcin in the serum (Menon et al, 1987).

There is a diurnal variation in BGP with peak levels occuring in the late night and early morning (Taylor et al, 1987) and it is therefore important to consider the time of the sampling.

2.4.2.2 Bone Resorption

Urinary excretion of calcium and hydroxyproline are both used as measures of bone resorption.

Urinary hydroxyproline:creatinine ratio

Hydroxyproline occurs almost exclusively in collagen, and urinary levels reflect collagen turnover. However hydroxyproline occurs in all collagen types, not just the type 1 collagen of bone. Furthermore dietary collagen contains hydroxyproline which is rapidly absorbed and excreted in urine. To prevent this as a source of error in hydroxyproline measurement, patients are placed on a collagen-free diet for 24 hours before the urine collection. Despite these drawbacks urinary hydroxyproline has been shown to be an accurate marker of bone resorption (Deacon et al, 1987; Nordin and Polley, 1987).

Hydroxyproline excretion can be measured either from a 24 hour urinary collection or as a ratio to creatinine excretion in an early morning urine sample.

Urinary calcium:creatinine ratio

Urinary excretion of calcium is also thought to be a measure of bone resorption. Nordin and Polley (1987) have shown a good correlation between urinary calcium and urinary hydroxyproline excretion. Calcium excretion, as an indicator of bone resorption has to be measured in an early morning urine sample, this being the second sample voided after an over-night fast.

2.4.3 CHANGES IN OSTEOPOROSIS

The skeleton is continuously being remodelled with bone resorption being followed by bone formation. In normal young adults formation and resorption are tightly coupled and bone mass is maintained. Bone loss implies an uncoupling with a relative or absolute increase in resorption over formation. When this uncoupling occurs, an increase in bone turnover leads to increased bone loss.

Serum BGP is a specific marker of bone formation and can predict the histological changes in osteoporosis (Brown et al, 1984). Studies have shown that serum levels may be high, normal or low in osteoporosis (Brown et al, 1984) reflecting the variation in bone turnover rates seen in this disorder. BGP is a vitamin K-dependent protein (see above) and this has led to interest in the role of vitamin K in bone metabolism. Hart, Shearer, Klenerman et al (1985) showed that vitamin K_1 levels were significantly lower in a group of patients with recent osteoporotic fractures (within 48 hours) and in a group with previous osteoporotic fractures than in an apparently normal control group. However the control group were between 7 and 14 years younger than the other 2 groups. Furthermore as the diagnosis of osteoporosis had not been excluded in the control group, the difference in vitamin K_1 levels may have been an effect of fracture rather than a cause of osteoporosis.

Christiansen et al (1987) in a 2 year follow-up study of 178 women in their early menopause showed that raised levels of serum alkaline phosphatase, urinary calcium and urinary hydroxyproline could be used to predict those women who were rapid "bone losers". This study has suggested that the combination of a blood and urine test, as above, together with a body fat measure in the early postmenopausal period, can be used to identify the majority of women at high risk of osteoporotic fracture. However Hui et al (1989) have subsequently shown that fast losers in the early postmenopause frequently reduce their bone loss rate substantially in the ensuing 5 years, so casting doubt on the relevance of early postmenopausal bone loss to the problem of fractures.

In most studies plasma concentrations of PTH in patients with osteoporosis have been indistinguishable from those of age- and sex- matched controls (Deftos LJ, 1987). Studies on vitamin D levels have also tended to show no difference between patients with osteoporosis and appropriate controls. However Silverberg et al (1989) showed that the PTH response to hypocalcaemia was decreased in patients with osteoporosis compared to age-matched controls. There is an age-related diminution in renal responsiveness to PTH (Tsai et al, 1984) and this study suggested that normal older patients are able to counter this by secreting more PTH, while patients with postmenopausal osteoporosis seem unable to respond in this way.

The main skeletal effect of calcitonin is to inhibit bone resorption. This has led to speculation about the role of calcitonin deficiency in the pathogenesis of osteoporosis (Deftos LJ, 1987). Studies measuring basal levels of plasma calcitonin have failed to show any consistent difference between women with osteoporosis and normal, age-matched controls. Provocative tests using calcium infusions to stimulate calcitonin secretion have also failed to show consistent differences. Taggart et al (1982) showed an impaired calcitonin response to calcium infusion in women with osteoporosis whereas Tiegs et al (1985), who also used a hypercalcaemic stimulus, were unable to show any significant difference between the patient and control groups.

The importance of oestrogen deficiency in the pathogenesis of postmenopausal osteoporosis is now well recognised. However, its exact mechanism of action in bone metabolism is still uncertain (see above).

Oestrogen deficiency is also important in the pathogenesis of bone loss in other situations (chapter 2.2.1), including athletic amenorrhoea. So far very little work has been done investigating the bone biochemistry changes that may occur in this condition (chapter 2.3).

2.5 MANAGEMENT OF OSTEOPOROSIS.

There are 3 important strategies in the management of osteoporosis; these are calcium supplementation, exercise and drug therapy.

2.5.1 CALCIUM SUPPLEMENTATION

The evidence for the benefits of calcium supplementation is limited (chapter 2.2.3). High calcium intake may marginally reduce the risk of fracture in postmenopausal women and may also enhance the peak bone mass achieved in younger women. However the value of calcium supplements has been seriously questioned in a recent review by Kanis and Passmore (1989).

2.5.2 EXERCISE

Aloia, Cohn, Ostuni et al (1978), Smith, Reddan et al (1981), Krolner et al (1983) and Chow, Harrison and Notarius (1987) have all demonstrated beneficial effects of exercise intervention in postmenopausal women (chapter 2.2.2). However the type of exercise that exerts the greatest benefit is uncertain.

Weight-bearing exercise seems important and there is evidence to suggest that even the activity of walking may increase BMD (Zylstra et al, 1989).

Exercise may also be of value in younger people by increasing peak bone mass but it may become counter-productive in women if it also leads to amenorrhoea (chapter 2.3).

2.5.3 DRUG THERAPY

2.5.3.1 Oestrogens

There is now substantial evidence confirming the protective effect of oestrogens in postmenopausal women. Studies have shown beneficial effects on both bone mineral density (Lindsay, Hart, Aitken et al, 1976; Horsman et al, 1977; Recker et al, 1977) and fracture incidence (Weiss et al, 1980). Oestrogen acts mainly by reducing bone resorption with a subsequent slow reduction in bone formation. The minimum effective dose appears to be 0.625 mg/day of conjugated equine oestrogen or its equivalent (Lindsay, Hart and Clark, 1984). There is also evidence to suggest that oestrogen therapy must be started within 3 years of the onset of the menopause for significant protection to occur (Lindsay, Hart, Aitken et al, 1976).

There are also other potential benefits of oestrogen therapy including a reduction in incidence of ischaemic heart disease and cerebrovascular accidents (Belchetz P, 1989). However the incidence of breast cancer may be increased (Bergkvist et al, 1989).

2.5.3.2 Calcitonin

Calcitonin inhibits bone resorption by direct action on osteoclasts. There is accumulating data on the use of calcitonin in the management of postmenopausal osteoporosis (Mazzuoli et al, 1986). This treatment seems to have a low incidence of serious side effects although there is some uncertainty about the dose and the best method of administration (Overgaard et al, 1989).

2.5.3.3 Fluoride

Bernstein et al (1966) showed a lower prevalence of fractures in areas with high fluoride concentrations in the drinking water and this together with the radiological features of skeletal fluorosis has stimulated interest in the use of fluoride as a treatment for osteoporosis. Fluoride has an anabolic effect on trabecular bone (Hedlund and Gallagher, 1987) but there are doubts about the quality of the new bone laid down. To provide good mineralization it is necessary to combine fluoride with calcium supplementation. Riggs, Seeman et al (1982) showed that

in postmenopausal osteoporosis, those women treated with fluoride in addition to conventional therapy had a lower incidence of fracture. However the groups being compared were unmatched so this conclusion has been challenged. Mamelle et al (1988) also showed a lower incidence of vertebral fractures in patients treated with fluoride compared to conventional therapy. There does seem to be a significant incidence of side effects with this treatment involving the upper GI tract and musculoskeletal pain in the lower limb. However its effect on bone formation makes this form of treatment promising.

2.5.3.4 Vitamin D

Although it increases calcium absorption from the gut, there is no convincing evidence that vitamin D alone exerts any therapeutic effect in the treatment of uncomplicated osteoporosis. In fact there is some evidence to suggest that when given in high dose it may even be harmful (Nordin, Horsman, Crilly et al, 1980).

2.5.3.5 Parathyroid hormone (PTH)

Although the administration of PTH in high dose predominantly results in bone resorption, there is evidence to suggest that when given chronically and in low dose it exerts an anabolic

effect on bone (Reeve et al, 1980). It seems to work more effectively with the concurrent administration of 1,25 $(OH)_2$vit D (Rosenthal et al, 1987).

2.5.3.6 Others

Anabolic steroids are known to increase bone mass (Geusens and Dequeker, 1986) but because of the high incidence of side effects in young women their use has been restricted generally to the elderly. They appear to act by reducing bone resorption. Diphosphonates inhibit bone resorption (Genant et al, 1987) but there use in the management of osteoporosis is still experimental.

The evidence for the therapeutic effects of the above treatments is based on research mainly in postmenopausal osteoporosis. There is very little information on therapeutic strategies for the management of osteoporosis in amenorrhoeic, premenopausal women. Oestrogen replacement would seem to be the obvious choice, but the dose required and the most appropriate method of administration have not yet been determined.

2.6 CONCLUSIONS.

We still know little about the cause of athletic amenorrhoea. Although the whole hypothalamic-pituitary-ovarian axis is involved, the factors influencing the hypothalamus are ill-defined. We also do not know much about the incidence in different sports.

Although it is well recognized that oestrogen exerts a protective effect on bone we do not understand how it interacts with other hormones involved with bone metabolism. Very little work has been done investigating these interactions in amenorrhoeic athletes.

We are also uncertain about the risk of developing premature osteoporotic fractures in these athletes. The high levels of exercise may provide some protection but at present we are also unclear about the type of exercise that is most effective.

There are only limited data available on the interaction of exercise, oestrogen status and calcium intake and its effect on peak bone mass in young, adult women.

CHAPTER 3: METHODS

3.1 AIMS OF THE STUDY.

The aims of the study were as follows :-

1. To determine the incidence of menstrual abnormalities amongst elite athletes in several popular sports in the U.K.

2. To confirm the findings of other cross-sectional studies that have shown decreased bone mineral density (BMD) in amenorrhoeic athletes compared to their eumenorrhoeic counterparts.

3. To determine the effect of different types of exercise on bone mineral density.

4. To determine the effect of dietary calcium on bone mineral density.

5. To investigate the effect of oestrogen on other biochemical parameters that are thought to be involved in bone metabolism.

6. To determine the longitudinal effects of different menstrual patterns and of different exercise patterns on bone mineral density.

3.2 STUDY DESIGN.

Subjects were selected from 3 different sports (chapter 3.3). On each visit to the British Olympic Medical Centre (BOMC) the menstrual pattern of each subject was defined. We were therefore able to investigate the effects of different menstrual patterns and of different types of exercise on bone mineral density.

Bone mineral density of spinal trabecular bone was measured on each subject using Computerized Tomography. Dual Photon Absorptiometry was used to measure cortical bone in the mid-shaft of the right femur and Dual Energy X-ray Absorptiometry for the bone density of the proximal femur (chapter 3.4).

The project was approved by the Harrow Health Authority Ethical Committee (Submission number 1448).

3.2.1 CROSS-SECTIONAL STUDY

In addition to the measurements of bone mineral density, several other parameters were investigated cross-sectionally. These can be classified into 3 main categories.

1. Measurements of Anthropometric and Physiological Parameters.
    (a) Height and weight
    (b) Body fat content

(c) Aerobic fitness - maximum oxygen uptake ($\dot{V}O_2max.$)

(d) Isokinetic back strength.

2. Measurement of Biochemical Parameters.

(a) Serum calcium, phosphate and albumin

(b) Serum alkaline phosphatase and osteocalcin

(c) Serum parathyroid hormone and 25-hydroxyvitamin D

(d) Urinary calcium, creatinine and hydroxyproline.

3. Measurement of Nutritional Intake.

(a) Daily calcium intake.

3.2.2 LONGITUDINAL STUDY

Each subject was followed up for a period of one year with measurements of bone mineral density (chapter 3.4) at the beginning and end of this interval. The following measurements were also repeated at the end of one year:-

(a) Height and weight

(b) Level of body fat

(c) Daily calcium intake.

Each subject was given a diary to keep a record of their menstrual pattern throughout the year of the study.

3.3 SELECTION OF SUBJECTS.

A questionnaire was designed to provide information on the menstrual patterns of high class female athletes from various sports. The final section asked whether the individual was prepared to be involved, as a subject, in the study.

3.3.1 SPORTS SELECTED FOR THE STUDY

The questionnaire, together with a covering letter, was distributed to National Squad athletes in several popular sports in Great Britain. The Governing Bodies for each of these sports assisted in distributing the questionnaires which enabled us to determine the response rates. There were 2 rowing categories, Lightweight (LW) and Heavyweight (HW). The Lightweights have to be at or below 59 Kg in order to compete whereas the Heavyweights are usually well above this level. We received responses from several sports. Table 4.1 lists these sports and the associated response rate.

The questionnaire was sent to 4 Ballet Companies (the Royal, the Festival, Sadlers Wells Royal and the Central School). There were 77 responses. It was not possible to define the response rate in this group. The questionnaire was also sent to 22 endurance runners (i.e. 1500 metres upwards). They were either in the national squad or were marathon runners with a personal best time

of less than 3 hours. These athletes had expressed an interest in being involved in the study and there was therefore a 100% response rate to the questionnaire.

There was a total of 226 replies to the questionnaire. From these responses a selection of athletes, willing to participate in the study, was made based on the following criteria :-

1. Sports in which there was a high degree of interest in being involved in the study;

2. Sports in which the majority of the athletes lived within easy reach of the hospital;

3. Sports in which approximately 50% of the athletes had exercise-induced menstrual abnormalities;

Ballet, Rowing and Running conformed to these criteria. Subjects from these 3 sports were contacted and those who were either amenorrhoeic, eumenorrhoeic or on the oral contraceptive (see below) and agreed to participate, were entered into the study. The original group consisted of the following:-

DANCERS	10
ROWERS	36
RUNNERS	21.

3.3.2 DEFINITIONS OF MENSTRUAL PATTERN

1. Amenorrhoea: No more than one period in the previous 6 months, or no more than 3 periods in the last year.

2. Oligomenorrhoea: Periods with a cycle length of greater than 35 days in women who are not amenorrhoeic.

3. Eumenorrhoea: Periods with a cycle length of less than 35 days.

4. Athletes who were taking the oral contraceptive formed a separate group.

5. Normal Oestrogen Status: athletes who were either eumenorrhoeic or on the oral contraceptive.

Based on this, the original group of athletes consisted of the following:-

25 Amenorrhoeics
27 Eumenorrhoeics
15 Oral Contraceptive Takers.

Table 3.3 gives the breakdown of the subjects by sport and by menstrual status.

TABLE 3.3 : NUMBER OF SUBJECTS INVOLVED IN ORIGINAL STUDY BY SPORT AND MENSTRUAL STATUS.

	AMENORRHOEICS	EUMENORRHOEICS	ORAL CONTRACEPTIVE TAKERS	TOTAL
DANCERS	4	5	1	10
ROWERS	10	15	11	36
RUNNERS	11	7	3	21
TOTAL	25	27	15	67

3.4 BONE DENSITY MEASUREMENTS.

Bone densitometry was performed at 3 skeletal sites, as follows:-

1. The bone mineral content (BMC) of the mid shaft of the right femur was measured using Dual Photon Absorptiometry (DPA).

2. The bone mineral content (BMC) of the left proximal femur was measured using Dual Energy X-Ray Absorptiometry (DEXA).

3. Spinal trabecular bone mineral density (TBD) of the second, third and fourth lumbar vertebrae was measured using Quantitative Computerized Tomography (QCT).

3.4.1 DUAL PHOTON ABSORPTIOMETRY (DPA)

A Novo BMC-Lab 22a dual photon absorptiometer was employed to measure BMC of the mid-shaft of the right femur, a site which consists almost entirely of cortical bone (Bohr and Schaadt, 1985). This scanner incorporates a radiation source of Gadolinium-153 (half life 280 days), which emits photons of approximately 44 KeV and 100 KeV. The source is housed in a shielded container below the couch and a highly collimated beam of these photons is used for scanning the supine patient. A collimated scintillation detector above the patient moves in synchronization with the radiation beam. The beam completes 20

transverse scans, each of 8 cm length with 2 mm steps between scans. This provides a complete measurement of a 4 cm length of the femoral mid-shaft. Scanning time is about 13 minutes.

Signals from the detector are evaluated in a 2 channel pulse height analyser. The amount of bone mineral in each slice is calculated automatically after correction for the soft tissue component. This value (in grams) when divided by the length of femur scanned (4 cm) gives the bone mineral content in g/cm of femur length, and when further divided by the mean bone diameter it gives the bone mineral content (BMC) in g/cm^2.

The coefficient of variation for these measurements is about 1.5% and the dose equivalent at the site of measurement is 0.1 mSv. (Matta et al, 1988).

3.4.2 DUAL ENERGY X-RAY ABSORPTIOMETRY (DEXA)

It is possible to make high precision measurements of the amount of bone mineral in the proximal femur using the relatively new technique of dual energy x-ray absorptiometry (DEXA). Proximal femur measurements were made on each subject using a Hologic QDR-1000 DEXA self-calibrating system. Instead of using a radioactive source as described for DPA, this uses a beam of x-rays which has components of 70 and 140 KVp. The subject lies supine with the feet placed in a jig designed to facilitate positioning of the hip region. The x-ray beam passes through the

subject at the level of the proximal femur and the signal is recorded by a synchronized detector unit, which in turn forms a rectilinear scan of the region. The maximum scanning area is 15.4 x 15.3 cm and the line spacing is 0.10 cm. Scanning time is about 7 minutes and the result is expressed in gm/cm^2 as for DPA (i.e. bone mineral content).

Since the data analysis facilities are relatively versatile, it is possible to obtain values of bone mineral density from the following regions of interest (figure 3.4.1):-

a) **FEMORAL NECK** - defined as a rectangular region 1.5 cm high and 6 cm or less wide where the bottom line is centred 0.75 cm below the narrowest area of the femoral neck.

b) **WARD'S TRIANGLE** - defined as a 1 cm^2 area of minimum bone density in the femoral neck region.

c) **GREATER TROCHANTER** - a triangular region whose boundaries are defined as the lateral edge of the femoral neck region and a line connecting the midpoint of the femoral midline to the point where the edge of the femur changes curvature below the trochanter.

These definitions are taken from the Hologic QDR-1000™ Operator's Manual, October 1989.

The maximum skin dose equivalent for these scans is about 0.04 mSv and the coefficient of variation is in the range of 1 to 3%.

3.4.3 QUANTITATIVE COMPUTERIZED TOMOGRAPHY (QCT)

QCT was used for measuring trabecular BMD of the lumbar spine based on the technique described by Cann and Genant (1980). A General Electric CT9000 was used to obtain 5 mm cuts through the centre of the second (L2), third (L3) and fourth (L4) lumbar vertebral bodies. The central area of each vertebra is made up almost entirely of trabecular bone. The beam conditions were 120 kV and 250 mAS with a scan time of 10 seconds. During scanning each subject lay flat over a crescent-shaped phantom that contained 5 solutions of K_2HPO_4 with mineral equivalents of 200, 100, 50, 0 and -50 mg/cm^3. These were used for calibration. Measurements of the largest circular area of trabecular bone were made, with care being taken to exclude areas of cortical bone. The area evaluated always exceeded 200 mm^2. Bone mineral density was then calculated using regression analysis of the phantom measurements. The average value of BMD for the 3 vertebrae was then calculated and expressed in mg/cm^3.

The coefficient of variation of the technique was about 3% (Cann and Genant, 1980) and the absorbed radiation dose was about 3 mSv in the abdominal region. Further consideration of this technique and its precision is given in the Appendix (page 280).

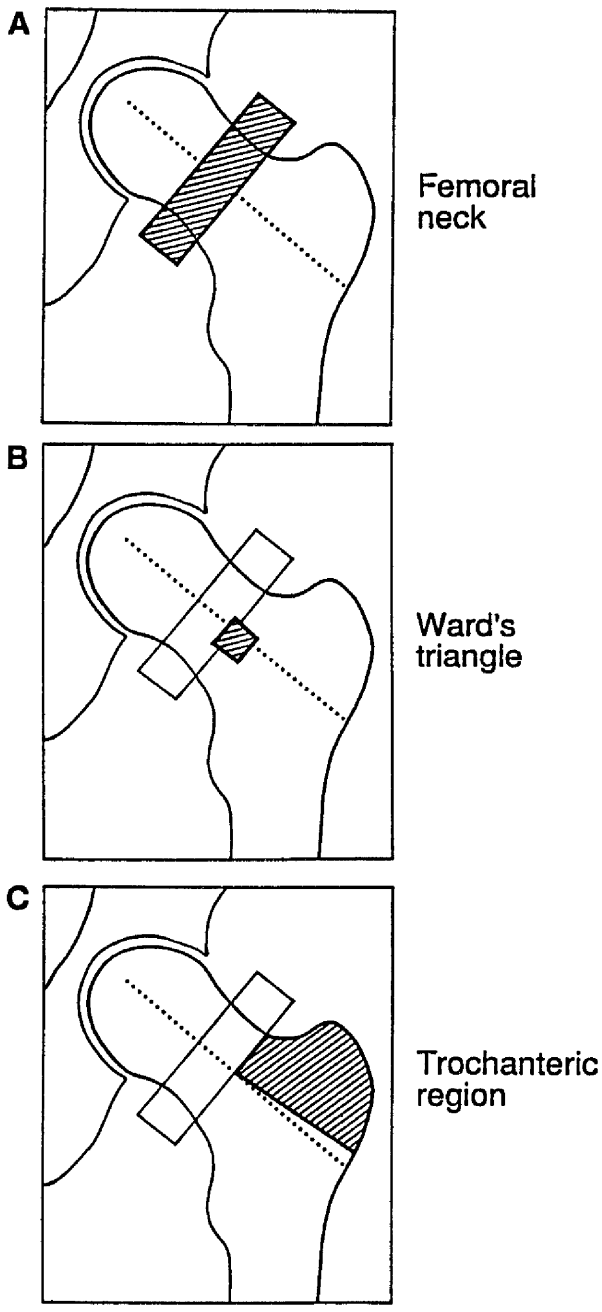

FIGURE 3.4.1:

Diagram of the Left Hip showing the Femoral Neck (A), Ward's Triangle (B) and the Trochanteric Region (C).

3.5 ANTHROPOMETRIC AND PHYSIOLOGICAL MEASUREMENTS.

The following anthropometric and physiological parameters were measured:-

3.5.1 Height and Weight

3.5.2 Body Fat

3.5.3 Aerobic Capacity

3.5.4 Back Strength.

3.5.1 HEIGHT AND WEIGHT

Using a Seca 713 stadiometer and platform balance scales, height (in cms) and weight (in kgs) were measured. From this body mass index (BMI) was calculated using the formula:-

$BMI = W/H^2$ where W = weight in Kgs and H = height in metres.

3.5.2 BODY FAT

Body fat was estimated using the technique validated by Durnin and Womersley (1974). Skinfold thickness was measured to the nearest mm using Harpenden calipers. These readings were made at four sites on each subject - biceps, triceps, subscapular and supra-iliac areas. They were measured on the right side of the body. The sum of these 4 measurements was then used to estimate

body fat as a percentage of body weight from the conversion table of Durnin and Womersley, who had shown a linear relationship between the logarithm of the sum of the 4 skinfold measurements and body density.

3.5.3 AEROBIC CAPACITY

Aerobic capacity ($\dot{V}O_2$ max) was measured using a Tunturi EL 400 cycle ergometer with electromagnetic braking. Continuous expired gas analysis was measured using the Jaeger EOS Sprint automated system which contained a paramagnetic oxygen analyser and an infrared carbon dioxide analyser. The heart rate (HR) was determined using a Rigel Cardiac Monitor 302 with the signal incorporated into the Jaeger printout.

A stepwise incremental protocol was used commencing at a load of 50 - 80 Watts and progressing by 30 Watt increments until volitional exhaustion. The criteria for achieving $\dot{V}O_2$max were either:-

a) HR within 5 beats of the age-related maximum HR (220 - age of patient) or,

b) an increase in load not accompanied by an increase in $\dot{V}O_2$ or,

c) a respiratory quotient value above 1.15.

3.5.4 BACK STRENGTH

Two indices related to back strength was assessed.

3.5.4.1 Psoas Cross-section at L3

With CT scanning Psoas cross-sectional area at the level of L3 was calculated. The tracer was used to outline the border of the psoas muscle bilaterally. The cross-sectional area of the muscle on each side was then measured directly using the scanner software and the average value of the 2 sides was taken.

3.5.4.2 Isokinetic Back Strength

Back strength was measured using a Lido isokinetic dynamometer, which was calibrated at regular intervals. With the subject in a seated position and the lower limbs strapped firmly to the base, flexion/extension movements were tested with the pivot point at the hip joint. Each subject was given time to become accustomed to the apparatus by having at least 3 practice runs. During the test each subject was given verbal encouragement. Torques were recorded at a constant velocity of 80 deg/sec. For both flexion and extension the peak torque and the total work done in the range of movement (both measured in Newton-metres) were recorded.

3.6 BIOCHEMISTRY MEASUREMENTS.

The following biochemistry measurements were made:-

3.6.1 Plasma calcium, phosphate, albumin and alkaline phosphatase.

3.6.2 Urinary calcium, hydroxyproline and creatinine.

3.6.3 Serum 25 hydroxy-vitamin D.

3.6.4 Serum parathyroid hormone (PTH).

3.6.5 Serum osteocalcin.

All venous samples were taken between 9.00 and 10.00 am to reduce any effect of diurnal variation.

3.6.1 PLASMA CALCIUM, PHOSPHATE, ALBUMIN AND ALKALINE PHOSHATASE.

These were all measured in the department of Clinical Chemistry, Northwick Park Hospital using the Dacos Multi-channel analyzer.

Calcium concentration in the plasma was estimated by an automated cresophthaleine-complexone colorimetric method (Connerty et al, 1966). Plasma calcium values were corrected for the albumin level using a recommended formula (Editorial, Br Med J, 1977). Plasma phosphate concentration was estimated by a method involving the formation of phosphomolybdate (Daley et al, 1972). Albumin concentration was measured using a method in which it was bound

with bromocresol green to form a complex (Doumas et al, 1971). Alkaline phosphatase was estimated using p-nitrophenyl phosphate as a substrate (Bowers et al, 1975).

3.6.2 URINARY CALCIUM, HYDROXYPROLINE AND CREATININE.

For 24 hours prior to collecting the urine sample, each subject went on a "gelatin-free" diet. After an overnight fast and having discarded the first urine sample of the morning, the second urine sample was analyzed by the Clinical Chemistry department at Northwick Park Hospital.

Urinary calcium estimation was performed by atomic absorption spectophotometry. Urinary hydroxyproline concentration was determined spectrophometrically based on the method of Kivorikko et al (1967). Urinary creatinine was measured by the Jaffe reaction using alkaline picrate to form the colour. The urine concentrations of calcium and hydroxyproline (in mmol/l) were both expressed as a ratio relative to the creatinine concentration in the urine (Nordin BEC, 1978).

3.6.3 SERUM 25 HYDROXY-VITAMIN D.

This was assayed by a radio-labelled competitive protein-binding technique using a binding protein obtained from vitamin D deficient rat serum and based on the technique described by Preece et al (1974). Analysis was performed by the Clinical Chemistry department at Northwick Park Hospital.

3.6.4 SERUM PARATHYROID HORMONE.

The "Allegro" Intact PTH immunoassay system was used for measurement of the biologically intact 84 amino acid chain of PTH. This is a two-site immunoradiometric assay based on the method described by Kao et al (1982). Analysis was performed by the Clinical Chemistry department at Northwick Park Hospital. The intra-assay variation was about 2.5% and the inter-assay variation about 6.0%.

3.6.5 OSTEOCALCIN.

Having taken the venous sample the serum was separated and stored at -70°C. Analysis was then performed by the Bone Disease Research Group, Clinical Research Centre, Northwick Park Hospital.

In the serum Osteocalcin consists of 2 forms; the active (GLA) and the inactive (GLU) fractions (see chapter 2.4.2.1). By adding hydroxyapatite to the serum, the active fraction is removed and the remaining supernatant contains only the inactive fraction (Price et al, 1981).

Osteocalcin was measured by RIA using the "Incstar Osteocalcin ^{125}I RIA" Kit, based on the method described by Deftos, Parthemore and Price (1981). Osteocalcin was measured in both the serum and supernatant (after adding hydroxyapatite). The concentration of the active fraction in the serum was determined by subtraction ($[osteocalcin]_{serum} - [osteocalcin]_{supernatant}$). The intra-assay variation was less than 8% and the inter-assay variation less than 14%.

3.7 ASSESSMENT OF CALCIUM INTAKE.

Calcium intake was assessed by questionnaire as described by Nelson, Hague et al (1988). The questionnaire was constructed in the form of a computer software packet (Calquest 2.0) enabling each subject to answer the questions directly onto a personal computer (Amstrad PC1512 HD20) which also calculated the calcium intake. This method of assessing calcium intake has been shown to correlate closely with 2 recognized methods, namely 5-day duplicate diets and 7-day weighed, dietary inventories (R= 0.76 and R= 0.69 respectively).

CHAPTER 4: THE INCIDENCE OF MENSTRUAL ABNORMALITIES AMONGST ELITE ATHLETES.

4.1 INTRODUCTION

There is only limited information on the incidence of menstrual abnormalities in athletes (chapter 2.1). We have therefore investigated this disorder amongst elite athletes in several sports.

4.2 METHOD

A questionnaire on menstrual patterns was prepared (chapter 3.3). This included questions on the menarche, the current age and menstrual status and on the present height and weight. Between October 1987 and January 1988 the governing bodies of several sports in Great Britain were asked to distribute the questionnaire to all their female national squad members. Returns from Badminton, Cycling, Gymnastics, Hockey, Rowing and Swimming have been analysed. The rowers were divided into Lightweights (LW) and Heavyweights (HW). The questionnaire was also sent to 4 professional ballet companies and to a group of elite endurance runners.

The definitions of amenorrhoea, oligomenorrhoea and eumenorrhoea are given in chapter 3.3.2. Body Mass Index (BMI) was obtained from the formula W/(H x H) where W = weight in Kgs and H = height in metres. The data from athletes on the oral contraceptive were not included.

Differences in incidence of menstrual abnormalities between the sports was assessed using Chi-squared analysis. In the sports with a large number of returns one-way analysis of variance was used to assess the difference in the variables between the menstrual groups. LW and HW rowers were compared using the students two-tailed t-test for unpaired samples. $P<.05$ was considered significant.

4.3 RESULTS

There was a total of 226 replies to the questionnaire giving an overall response rate of 72%. Table 4.1 gives the response rates from each individual sport. There were 22 replies from the runners and 77 from the ballet dancers. In these sports we were unable to define the response rates as we received no information on the method of distribution.

The incidence of menstrual irregularity overall (amenorrhoea + oligomenorrhoea) was 52%. Table 4.2 and Figure 4.1 show the incidence which differed significantly between the sports ($p<.01$). For example, in hockey and badminton it was low but all

7 of the gymnasts questioned gave a history of menstrual irregularity. 5 of these were amenorrhoeic with 3, all aged 16, being pre-menarchal. The incidence was also significantly higher in the LW rowers than it was in the HW's ($P < .025$).

BMI was calculated for each menstrual group (amenorrhoea, oligomenorrhoea and eumenorrhoea) in each sport. Table 4.3 gives these results. BMI was lower in the amenorrhoeic dancers and runners than in their eumenorrhoeic counterparts ($p < .002$ and $p < .05$ respectively). However between rowers, swimmers and cyclists there was no significant differences. There were no data available for gymnasts. BMI was associated with menstrual pattern only in those sports with generally low values for BMI.

Amongst the rowers (LW and HW) and amongst the runners the age of menarche was similar in each of the menstrual groups (table 4.4). Furthermore in each of these 2 Sports there were no significant differences in current age between the menstrual groups (table 4.5). Amongst the dancers however, there were significant differences in age with the eumenorrhoeics being older than the other two groups ($p < .05$), and menarche which was significantly delayed in the amenorrhoeics ($p < .025$). For the other sports the groups were too small to draw any conclusions.

4.4 DISCUSSION

Athletic amenorrhoea was first recognised in the late 1970's (Dale et al, 1979). The cause is not completely understood but factors associated with its development have been well described (Noakes et al, 1988; Williams, 1984). Some of these factors may help to explain the difference in incidence between the sports. Endurance training forms a component of all the sports chosen and previous studies have shown a direct relationship between the intensity of training and the incidence of amenorrhoea (Feicht et al, 1978; Feicht Sanborn et al, 1982).

Runners and cyclists do the most intensive endurance training, running 60-80 miles/week or cycling 250-300 miles/week respectively. Hockey and badminton players tend to run only 10-20 miles/week as part of their training. This might help to account for the difference in menstrual abnormalities between athletes in these sports.

Calorie intake also seems to be a significant factor. An extreme example is anorexia nervosa where amenorrhoea develops almost invariably (Walsh, 1980). Calorie restriction is important in certain sports that require a high power-to-weight ratio such as ballet, cycling, gymnastics and long distance running. In rowing calorie restriction is only significant in those who have to "make the weight". This might account for the higher incidence of menstrual abnormalities amongst LW rowers ($p < .025$) even though their training methods are similar to the HW's.

The combination of intense training and calorie restriction will cause weight loss and a reduction in body fat which are features seen in these athletes and which may also be important in the pathophysiology of amenorrhoea (Noakes et al, 1988). Some previous studies have shown a difference in body fat levels between amenorrhoeic and eumenorrhoeic athletes (Schwartz et al, 1981; Glass et al, 1987) suggesting a minimum level of body fat necessary for the onset and maintenance of the menstrual cycle. A critical level of body fat was first proposed by Frisch and McArthur (1974). However, others have shown no such difference between the menstrual groups (Sanborn et al, 1987). The discrepancy might be explained first by the differences in selection criteria in each study and second by the different methods employed to measure body fat (Cumming and Rebar, 1984). At present the relationship between low body fat and abnormal menstrual pattern is not fully understood.

In this study there are no data on body fat levels, as measured using one of the standard methods (Cumming and Rebar, 1984). However a rough index of "body fatness" is given by measuring the ratio of weight to height (Body Mass Index). Even though this only approximates with percentage body fat, we have shown significantly lower BMI's in those athletes with menstrual irregularity in agreement with the theory of Frisch and McArthur (1974). This only applies to those sports with generally low

BMI's such as ballet and running. In sports where the BMI is higher other factors may play a more critical role in the development of amenorrhoea.

Current age and age at which training began are also important factors. Previous studies have shown that amenorrhoeic athletes are significantly younger than their eumenorrhoeic counterparts (Speroff et al, 1980). The reason for this is uncertain but one possibility is that the menstrual cycle may be more sensitive to the effects of exercise in the postmenarchal period than it is later on. There is also evidence to suggest that training in the premenarchal period is associated not only with delayed menarche but also with menstrual irregularity in later life (Frisch et al, 1981; Wakat et al, 1982). Thus studies have shown that amenorrhoeic athletes tend to have a later menarche than their eumenorrhoeic counterparts (Feicht et al, 1978).

The gymnasts were generally younger than the other athletes studied and also tended to have delayed menarche (3 gymnasts were still pre-menarchal). Only in the dancers did we find younger age and delayed menarche to be associated with menstrual irregularity. Both dancers and gymnasts take up their training well before the menarche and this is almost certainly a contributary factor. Such differences did not apply to the other sports and may reflect the older age that such athletes take up their sport.

The incidence of menstrual irregularity may have been overestimated because those athletes who have menstrual problems were perhaps more likely to respond to the questionnaire than those who have not. The high response rates will reduce this effect. The occurrence of menstrual problems in each sport is representative of some of the physiological requirements for that sport and it also helps to highlight some of the mechanisms behind the development of amenorrhoea.

TABLE 4.1 THE RESPONSE RATES TO THE QUESTIONNAIRE.

	NUMBER OF RESPONSES	NUMBER SENT	RESPONSE RATE (%)
BADMINTON	9	15	60
CYCLING	16	20	80
GYMNASTICS	8	12	67
HOCKEY	15	23	65
ROWING-LW	34	45	76
ROWING-HW	24	40	60
SWIMMING	21	27	78

TABLE 4.2 INCIDENCE OF MENSTRUAL ABNORMALITIES IN EACH SPORT (%).

	AMENORRHOEA (AMEN)	OLIGOMENORRHOEA (OLIGO)	AMEN + OLIGO	EUMENORR -HOEA
BADMINTON	-	-	-	100
CYCLING	30	40	70	30
DANCING	27	24	52	48
GYMNASTICS	71	29	100	-
HOCKEY	-	17	17	83
ROWING-HW	-	33	33	66
ROWING-LW	46	21	67	33
RUNNING	45	20	65	35
SWIMMING	-	31	31	69

TABLE 4.3 MEAN (SD) BMI (Kg/m^2) FOR EACH MENSTRUAL GROUP IN EACH SPORT.

	AMENORRHOEA (AMEN)	OLIGOMENORRHOEA (OLIGO)	AMEN + OLIGO	EUMENORRHOEA
BADMINTON	-	-	-	23 (1.8)
CYCLING	21 (1.9)	20.4 (3.1)	20.7 (2.5)	20.1 (2.6)
DANCING	17.7 (1.2)	17.8 (0.9)	17.7 (1.0)	19.3 (1.1)
GYMNASTICS	-	-	-	-
HOCKEY	-	22.5 (1.5)	22.5 (1.5)	22.7 (1.3)
ROWING-HW	-	23 (0.7)	23 (0.7)	22.8 (1.4)
ROWING-LW	20.8 (1.7)	20.4 (0.9)	20.7 (1.5)	21.4 (1.3)
RUNNING	18.3 (0.8)	20.2 (2.0)	18.9 (1.5)	19.4 (1.2)
SWIMMING	-	20.2 (0.6)	20.2 (0.6)	20.8 (1.0)

TABLE 4.4 MEAN (SD) AGE OF MENARCHE (yrs) FOR EACH MENSTRUAL GROUP IN EACH SPORT.

	AMENORRHOEA (AMEN)	OLIGOMENORRHOEA (OLIGO)	AMEN + OLIGO	EUMENORRHOEA
BADMINTON	-	-	-	14.4 (2.1)
CYCLING	15.3 (2.1)	14.8 (0.5)	15 (1.3)	13.3 (2.5)
DANCING	15.5 (2.3)	14.6 (0.8)	15.1 (1.8)	14 (1.8)
GYMNASTICS	15.8 (1.3)	16	15.9 (1.1)	-
HOCKEY	-	14	14	14.1 (1.0)
ROWING-HW	-	13.5 (1.8)	13.5 (1.8)	13.7 (0.9)
ROWING-LW	13.5 (0.9)	13.4 (1.5)	13.4 (1.1)	13.6 (1.2)
RUNNING	13.4 (0.7)	14.3 (2.1)	13.7 (1.3)	13.8 (1.4)
SWIMMING	-	13.8 (1.8)	13.8 (1.8)	14.2 (1.3)

TABLE 4.5 CURRENT MEAN (SD) AGE (yrs) FOR EACH GROUP IN EACH SPORT.

	AMENORRHOEA (AMEN)	OLIGOMENORRHOEA (OLIGO)	AMEN + OLIGO	EUMENORRHOEA
BADMINTON	-	-	-	22 (3.4)
CYCLING	26.3 (6.0)	30.8 (3.1)	29.8 (4.1)	26.3 (4.9)
DANCING	18.7 (2.0)	19.7 (2.6)	19.1 (2.3)	21.9 (5.1)
GYMNASTICS	16.6 (1.1)	19.5 (0.7)	17.4 (1.7)	-
HOCKEY	-	22 (1.4)	22 (1.4)	22.8 (2.7)
ROWING-HW	-	23 (2.6)	23 (2.6)	24.2 (2.8)
ROWING-LW	24.6 (1.1)	27.2 (2.8)	25.4 (3.7)	26.6 (5.5)
RUNNING	26 (2.7)	27 (2.6)	26.3 (2.6)	26.3 (5.5)
SWIMMING	-	19.8 (2.4)	19.8 (2.4)	20.2 (4.2)

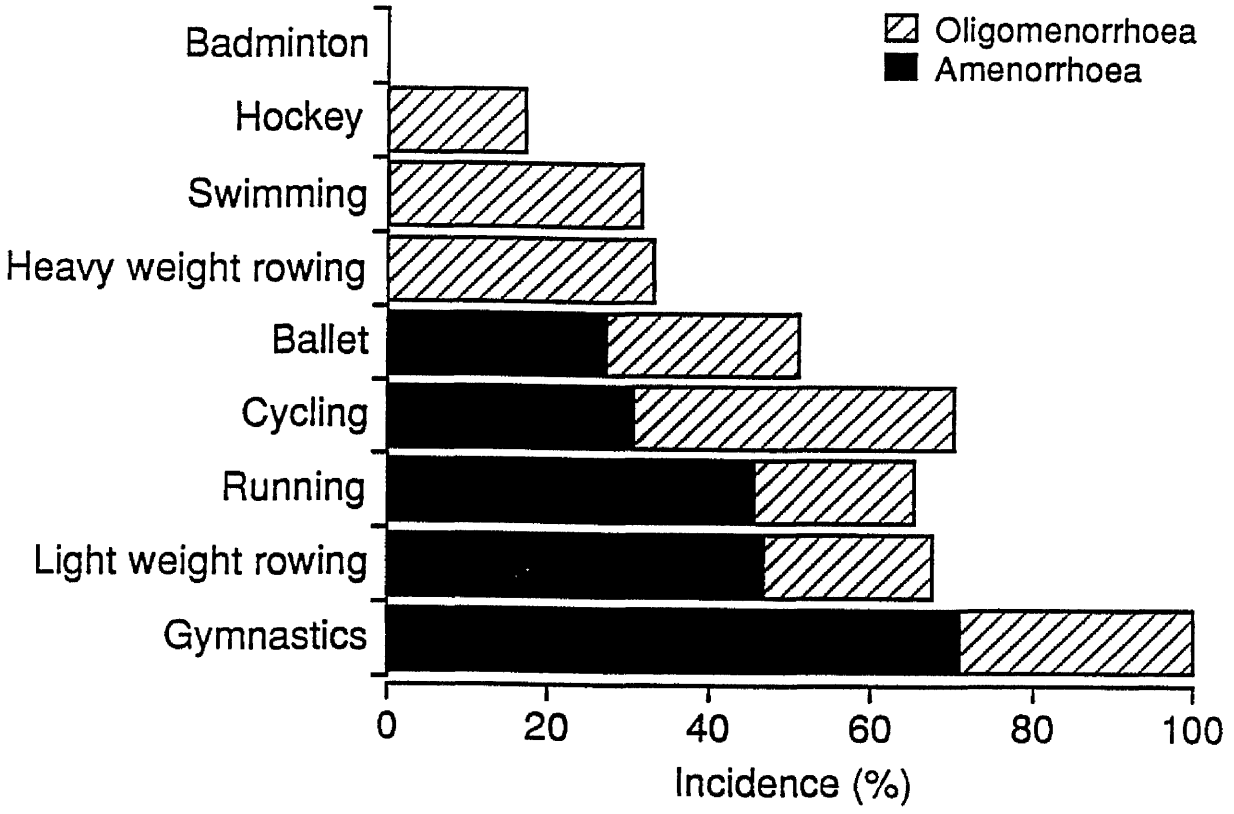

FIGURE 4.1:

Incidence of Menstrual Abnormalities in Each Sport.

CHAPTER 5: THE EFFECT OF OESTROGEN STATUS ON BONE MINERAL DENSITY

5.1 INTRODUCTION

Previous work has shown that athletes with amenorrhoea have decreased bone mineral density compared to their eumenorrhoeic counterparts (chapter 2.3). This reduction occurs at sites of trabecular bone, but not in cortical bone of the upper limb.

A study by Marcus et al (1985) showed that in amenorrhoeic athletes, the effect of high levels of exercise on increasing BMD was not sufficient to compensate for losses due to low oestrogen status but the cohort was small. Oestrogen-containing oral contraceptives provide one possible way of preventing mineral loss due to amenorrhoea but there is very little information on their effect on BMD.

We have therefore measured the BMD in cortical and trabecular bone in athletes with amenorrhoea, eumenorrhoea and those on the oral contraceptive and compared them with a group of eumenorrhoeic, sedentary controls.

5.2 METHOD

Sixty-seven athletes were investigated and consisted of 10 dancers, 36 rowers and 21 runners (chapter 3.3). This group contained 25 with amenorrhoea, 27 with eumenorrhoea and 15 on the oestrogen-containing oral contraceptive (chapter 3.3).

The bone mineral content (BMC) of the mid-shaft of the right femur, which consists predominantly of cortical bone, was measured using dual photon absorptiometry (chapter 3.4.1). The trabecular bone mineral density (TBD) of the second, third and fourth lumbar vertebrae was measured using CT scanning (chapter 3.4.3). These measurements were performed in all 67 athletes.

The BMC and TBD measurements as described above were also performed on a group of 13 eumenorrhoeic, sedentary women of a similar age. Their results were used as control data for comparison with the athletes in each menstrual group.

The results in the eumenorrhoeic sedentary controls (SED) and the amenorrhoeic (AMEN), eumenorrhoeic (EUMEN) and the oral contraceptive-taking (OC) athletes were compared using one-way analysis of variance.

5.3 RESULTS

Tables 5.1-5.4 give the age, height, weight and bone density measurements for each subject from each of the 4 groups.

Table 5.5 summarizes the mean values for lumbar spine and femoral shaft bone density measurements in each of the 4 groups. TBD in the lumbar spine was significantly lower ($P<0.0001$) in the amenorrhoeic athletes (168 mg/cm^3) than in their eumenorrhoeic (211 mg/cm^3) and oral contraceptive-taking counterparts (215 mg/cm^3). TBD in the sedentary control group (187 mg/cm^3) lay midway between these groups (figure 5.1).

Cortical BMC in the femoral mid-shaft (table 5.5 and figure 5.2) was 1.45 gm/cm^2 in the amenorrhoeic athletes with similar levels in their eumenorrhoeic (1.45 gm/cm^2) and oral contraceptive-taking (1.46 gm/cm^2) counterparts. BMC was lower in the sedentary control group (1.40 gm/cm^2) but this was not statistically significant ($P = 0.38$).

5.4 DISCUSSION

Drinkwater et al (1984), using dual-photon absorptiometry, demonstrated that amenorrhoeic athletes have reduced bone mineral density in the lumbar spine compared to their eumenorrhoeic counterparts. They also showed that the mineral content in the forearm at sites of predominantly cortical bone was similar in

the 2 groups studied. Marcus et al (1985) and Nelson et al (1986) also reported reductions in trabecular bone with preservation of cortical bone density in the upper limb. The numbers involved in these 3 studies (28, 17 and 28 respectively) were small compared to ours. We have confirmed that spinal trabecular bone is reduced in amenorrhoeic athletes. We have also shown that cortical bone in the lower limb is not affected by low oestrogen status.

Marcus et al (1985) showed that the trabecular bone density in the amenorrhoeic athletes was lower than in a eumenorrhoeic, sedentary control group. We have also shown that amenorrhoeic athletes have lower trabecular bone densities than eumenorrhoeic, sedentary controls. This suggests that high levels of exercise in general are not sufficient to compensate for the low oestrogen status. In Marcus's study the amenorrhoeic group consisted of 11 athletes all of whom were runners. However our group of 25 amenorrhoeic athletes contained 10 rowers. The trabecular bone density in the spine in this subgroup was similar to the level in the control group suggesting that specific upper body exercise might be sufficient to compensate the effects of low oestrogen status (chapter 6).

Both cortical and trabecular bone density levels were similar in the eumenorrhoeic and oral contraceptive-taking athletes. In all the oral contraceptive-takers, the oestrogen was in the form of ethinyloestradiol. This suggests that the oestrogen-containing oral contraceptive has an effect on bone density similar to endogenous oestrogen.

5.5 CONCLUSIONS

a) Low oestrogen status reduces trabecular but not cortical bone density.

b) Exercise does not completely compensate for the effects of amenorrhoea on trabecular bone mineral density.

c) Exercise has its greatest effect on increasing bone density when normal oestrogen status is preserved.

d) Women taking the oral contraceptive and those with eumenorrhoea have similar bone density values.

TABLE 5.1
DEMOGRAGHIC AND BONE DENSITY MEASUREMENTS IN THE 25 AMENORRHOEIC ATHLETES.

Reg. No	AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)	LUMBAR SPINE TBD (mg/cm^3)	FEM.SHAFT BMC (gm/cm^2)
006	30	168	60	188	1.32
007	27	168	57	216	1.49
008	30	166	62	154	1.34
014	22	173	62	176	1.20
019	22	171	60	191	1.59
023	20	161	43	168	1.34
024	20	174	53	185	1.29
026	22	165	49	115	1.27
027	22	175	61	160	1.47
028	21	163	54	200	1.45
031	18	171	56	121	1.31
035	26	168	58	197	1.39
042	21	164	50	144	1.46
043	26	161	51	221	1.62
046	27	164	47	148	1.29
052	25	156	44	149	1.61
060	20	167	49	221	1.58
061	21	161	63	191	1.69
062	25	170	54	198	1.63
063	27	162	48	96	1.44
064	27	164	47	105	1.48
065	28	163	49	226	1.45
066	22	160	48	115	1.46
068	28	155	46	149	1.48
069	28	153	43	158	1.50

TABLE 5.3
DEMOGRAPHIC AND BONE DENSITY MEASUREMENTS IN THE 15 ORAL CONTRACEPTIVE-TAKING ATHLETES.

Reg. No	AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)	LUMBAR SPINE TBD (mg/cm^3)	FEM.SHAFT BMC (gm/cm^2)
003	25	165	59	256	1.42
004	25	176	70	252	1.31
005	23	171	59	252	1.50
010	28	171	60	234	1.44
012	25	175	63	221	1.46
015	31	174	66	239	1.45
022	25	167	60	185	1.41
030	21	159	52	235	1.42
034	25	171	59	212	1.49
044	23	182	73	209	1.48
045	21	172	65	153	1.52
047	27	164	50	178	1.48
049	24	177	57	224	1.62
050	29	157	47	140	1.39
057	24	161	47	215	1.52

TABLE 5.2

DEMOGRAPHIC AND BONE DENSITY MEASUREMENTS IN THE 27 EUMENORRHOEIC ATHLETES.

Reg. No	AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)	LUMBAR SPINE TBD (mg/cm^3)	FEM.SHAFT BMC (gm/cm^2)
001	22	183	78	193	1.39
002	23	183	74	276	1.44
009	20	174	68	242	1.41
011	26	165	59	181	1.23
016	25	179	77	187	1.48
017	27	168	56	210	1.41
018	26	169	70	184	1.55
020	31	171	63	196	1.59
021	25	184	76	198	1.46
025	20	171	64	196	1.40
032	25	159	55	238	1.43
033	24	173	69	199	1.50
036	25	171	57	204	1.31
037	27	165	50	199	1.42
038	22	162	55	187	1.38
039	27	169	56	283	1.41
040	24	167	49	214	1.45
048	29	163	53	178	1.41
051	30	167	51	223	1.68
053	22	160	46	247	1.44
054	23	168	65	210	1.55
055	30	159	50	206	1.35
056	20	165	58	180	1.39
058	29	169	66	224	1.47
059	26	172	61	266	1.53
067	28	170	55	168	1.49
070	25	167	58	197	1.48

TABLE 5.4

DEMOGRAPHIC AND BONE DENSITY MEASUREMENTS IN THE 13 EUMENORRHOEIC, SEDENTARY CONTROLS.

AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)	LUMBAR SPINE TBD (mg/cm^3)	FEM.SHAFT BMC (gm/cm^2)
26	170	73	154	1.48
28	157	64	172	1.29
26	163	56	209	1.44
37	160	54	214	1.51
25	160	53	186	1.35
26	156	48	195	1.37
22	157	45	190	1.41
23	167	53	160	1.46
32	157	55	151	1.41
32	157	53	212	1.26
27	167	57	181	1.35
28	157	51	169	1.19
31	158	52	234	1.62

TABLE 5.5 : MEAN (95% CONFIDENCE INTERVAL) BONE DENSITY FOR EACH GROUP.

	SED	AMEN	EUMEN	OC
LUMBAR SPINE (mg/cm^3)	187 (168,205)	168 (155,181)	211 (198,223)	215 (198,232)
FEMORAL SHAFT (g/cm^2)	1.40 (1.34,1.45)	1.45 (1.40,1.49)	1.45 (1.41,1.49)	1.46 (1.41,1.52)

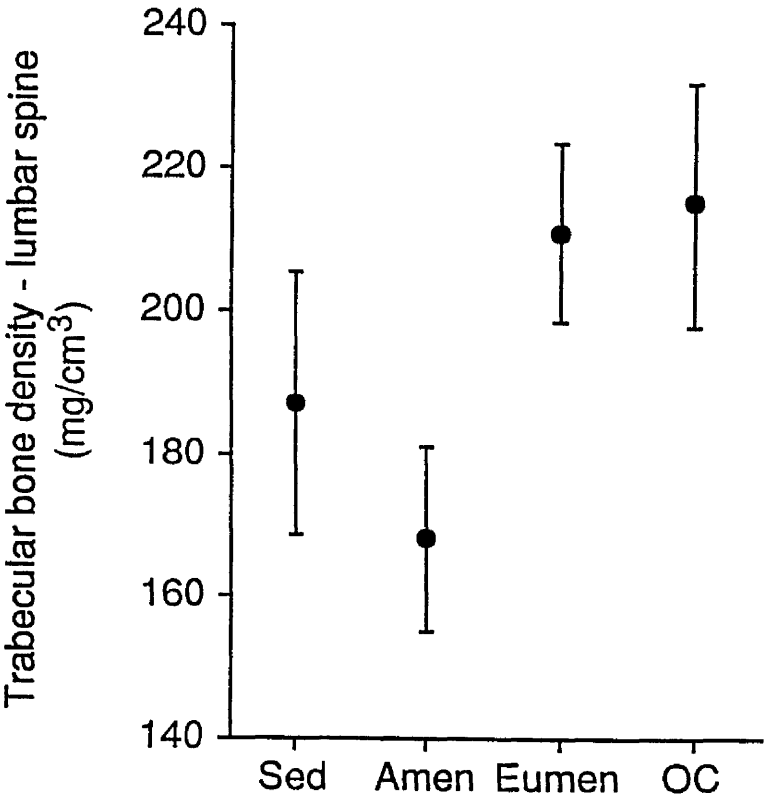

FIGURE 5.1

Spinal Trabecular BMD in the Sedentary Controls and the Amenorrhoeic, Eumenorrhoeic and Oral Contraceptive-taking Athletes.

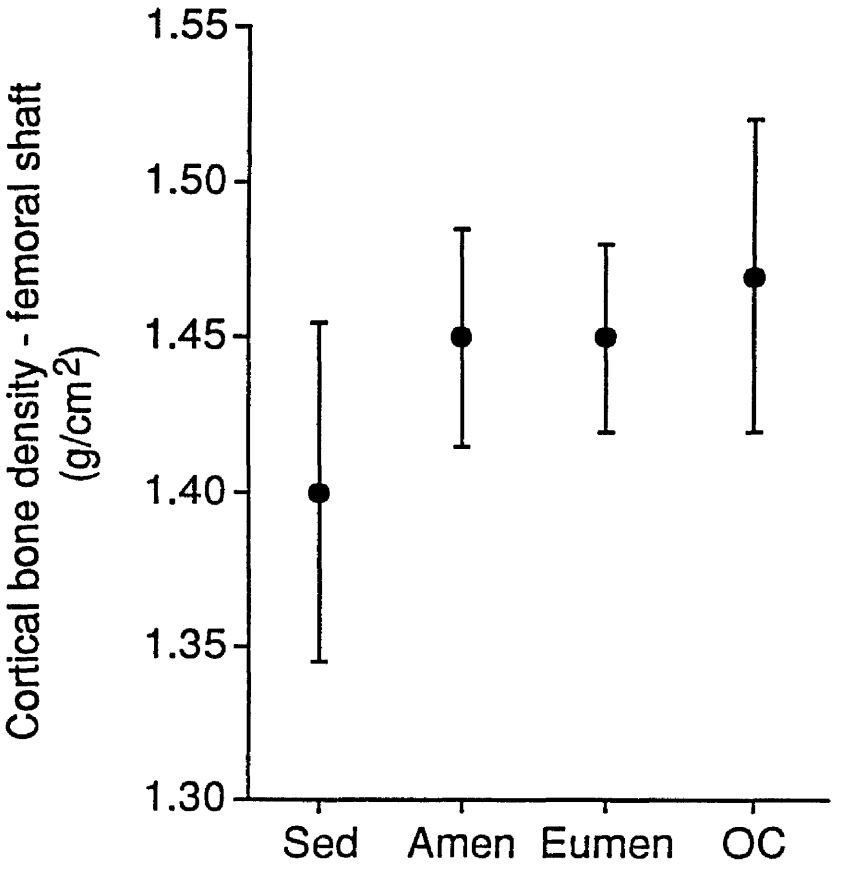

FIGURE 5.2:

Cortical BMD in the Sedentary Controls and in the Amenorrhoeic, Eumenorrhoeic and Oral Contraceptive-taking Athletes.

CHAPTER 6: EFFECTS OF SPORTING ACTIVITY ON BONE MINERAL DENSITY.

6.1 EFFECT OF ROWING ON THE TRABECULAR BONE DENSITY OF THE SPINE.

6.1.1 INTRODUCTION

Physical activity has been shown to increase bone strength in both animal (chapter 2.2.2.5) and human studies (chapter 2.2.2). In elite athletes, studies have shown increased bone density in the wrists of the playing arm of tennis players and in the os calcis of runners (chapter 2.2.2.2) suggesting that exercise exerts its effect mainly on the bones at sites of maximum stress. Intense endurance training in females can lead to amenorrhoea which in turn has been shown to reduce the trabecular bone mineral density (BMD), especially in the lumbar spine (chapter 2.3). However previous studies have tended to focus on athletes who concentrate mainly on lower body exercise.

Rowing is a sport that involves considerable back exercise. There is also a high incidence of amenorrhoea amongst the lightweight competitors. There has been one previous study of lightweight female rowers (Snyder et al, 1986) but the cohort was small and the results difficult to interpret. We have studied a large group of elite, lightweight women rowers and compared them with a group of elite, female athletes whose predominant exercise involves the

lower body, with about 50% in each group being amenorrhoeic. They have also been compared with a eumenorrhoeic, sedentary control group.

6.1.2 METHOD

Amongst the cohort of 67 athletes (chapter 3.3), there were 26 lightweight rowers of which 10 were amenorrhoeic and 9 eumenorrhoeic as defined in chapter 3.3. There were also 31 non-rowers consisting of 21 runners and 10 dancers. This group contained 15 amenorrhoeic athletes (11 runners and 4 dancers) and 12 with eumenorrhoea (7 runners and 5 dancers)- see table 3.3. We have studied these 4 groups - amenorrhoeic and eumenorrhoeic rowers, amenorrhoeic and eumenorrhoeic non-rowers - and also a group of 13 eumenorrhoeic, sedentary controls (chapter 5).

Trabecular bone mineral density of the lumbar spine (mg/cm^3) was measured by Quantitative Computed Tomography (QCT) using a General Electric CT9000 scanner (chapter 3.4.3).

Back strength was assessed both by estimating psoas muscle size and by isokinetic dynamometry. Psoas cross-sectional area at the level of L3 and isokinetic back strength at a constant velocity of 80 $deg.sec^{-1}$ were both measured (chapter 3.5.4).

Statistical Methods

Statistical analysis consisted of fitting a linear model (McCullagh and Nelder, 1983) to attempt to assess the effects of Sport (Rowers v Non-Rowers i.e. Dancers and Runners) and Menstrual Status (Amenorrhoea v Eumenorrhoea) on the lumbar spine BMD of the athletes. Height, weight and age were also considered in case they too exerted any significant effect.

The first stage was to determine which of these 5 variables on their own best explained the differences in the lumbar spine BMD. That variable was incorporated into the model. Then each one of the remaining variables was added to the model to assess which one of them explained the greatest additional variability (i.e. the one with the largest F ratio). That variable was then also incorporated into the model. This procedure was repeated until all remaining variables had a P-value (inversely related to the F ratio) for entering the model of greater than 0.05 (i.e. no additional significant effect on BMD). Finally the residuals were tested for normality using the Shapiro Wilk's W test (Royston, 1982) and for equal variances in categories defined by sport and/or menstrual status using the Schweder test (Schweder, 1981). 95% confidence intervals for the sports and/or menstrual states were constructed using the residual variance from final model fitted.

The sports were compared for back strength using one way analysis of variance after checking for normality and equal

variances as above.

6.1.3 RESULTS

Tables 6.1.1 and 6.1.2 give the results for the amenorrhoeic and eumenorrhoeic lightweight rowers respectively. Tables 6.1.3 and 6.1.4 give the results for the amenorrhoeic and eumenorrhoeic non-rowers respectively. Table 5.4 gives the results for the eumenorrhoeic, sedentary controls.

There were no significant differences between the dancers and the runners in any of the parameters investigated so they have been grouped together as non-rowers.

Comparison of the Rowers with the Sedentary Controls

Table 6.1.5 and figure 6.1.1 show the differences between the eumenorrhoeic, sedentary controls (SED) and the amenorrhoeic (AM) and eumenorrhoeic (EU) rowers. The trabecular bone density in the sedentary controls (187 mg/cm^3) and in the amenorrhoeic, lightweight rowers (186 mg/cm^3) was similar. In the eumenorrhoeic, lightweight rowers it was 215 mg/cm^3 which was significantly higher than in the other 2 groups ($P = 0.016$).

Comparison of the Rowers with the Non-rowers

Table 6.1.6 gives details of the demographic variables for the two sporting groups. The rowers were taller (means 169 v 164 cm) and heavier (59.8 v 51.3 Kg) than the non-rowers but their ages (25 yrs) were similar.

The bone mineral density in the eumenorrhoeic athletes was 210 mg/cm^3 whereas it was only 168 mg/cm^3 in the athletes with amenorrhoea (table 6.1.7). This difference was highly significant ($P = 0.0002$). In the rowers the bone mineral density was 199 mg/cm^3 compared to 178 mg/cm^3 in the non-rowers. This difference was also statistically significant ($P = 0.05$). Figure 6.1.2 shows the mean bone density measurements in each of the 4 groups (amenorrhoeic and eumenorrhoeic rowers and non-rowers).

Table 6.1.8 shows the results of the model fitting for lumbar spine TBD. Stage 1 shows the highly significant effect of menstrual status ($F = 17.08$; $P = 0.0002$), and the lesser effects of weight ($F = 4.70$) and sport ($F = 3.31$). However when menstrual status was taken into account (table 6.1.8, stage 2) weight no longer exerted an effect on bone mineral density ($F = 1.22$; $P = 0.28$) and in fact only sport had any significant effect ($F = 4.06$; $P = 0.05$). When both sport and menstrual status were incorporated (table 6.1.8, stage 3) into the model no other variable had a significant effect.

Table 6.1.8, stage 3 also shows that there was no significant interaction between the effects of sport and menstrual status ($F = 1.20$; $P = 0.28$) on bone mineral density. This shows that the significant effect of menstrual status applies equally to both sporting groups, and that the significant effect of sports status applies equally to both menstrual states.

Tables 6.1.9 and 6.1.10 give the back strength measurements for each rower and non-rower respectively. The rowers had greater psoas cross-section at L3 ($928mm^2$ v $780mm^2$) and greater isokinetic peak torques for both flexion (137Nm v 106Nm) and extension (214Nm v 174Nm) than the non-rowers (table 6.1.11). These differences were highly significant ($P = 0.006$, $P < 0.0001$, $P = 0.007$ respectively).

6.1.4 DISCUSSION

Previous studies have shown that physical activity increases bone mineral at the sites where the skeleton is maximally stressed (Huddleston et al, 1980; Williams et al, 1984; Pirnay et al, 1987). The importance of investigating female rowers is that not only do they have an increased risk of developing amenorrhoea but they also exercise that part of the skeleton known to be sensitive to the effects of low oestrogen status (chapter 2.2.1).

We have shown that trabecular bone mineral density of athletes with amenorrhoea is significantly lower than in those with normal menses and this applies equally to the different sporting groups. The amenorrhoeic rowers and the eumenorrhoeic, sedentary controls have similar levels of bone mineral density in the spine which does suggest that the type of exercise performed by the rowers on the spine may be sufficient to compensate the effects of low oestrogen status.

Using the technique of model fitting, menstrual status has the greatest effect on bone mineral density. Once menstrual status is incorporated into the model, the only other factor that accounts for additional variability in bone density is the sporting group (i.e. it is the type of activity performed by the rowers and not their increased weight and height that accounts for their higher bone mineral density levels). This effect is measurable regardless of menstrual status. However in chapter 8 we have also shown that spinal bone density is related to dietary calcium intake and that once this is incorporated into the model, the effect of Sporting activity disappears. This complex relationship between dietary calcium intake, Sporting activity and spinal bone density is discussed further in chapter 8.

Snyder et al (1986) studied bone mineral content of the lumbar spine using dual photon absorptiometry (DPA) in rowers and showed similar levels in 4 amenorrhoeic rowers, 7 eumenorrhoeic rowers and 9 eumenorrhoeic sedentary controls. These findings are surprising because if exercise were to exert an anabolic effect

and compensate for bone loss due to amenorrhoea, one might expect that the rowers with eumenorrhoea would have a higher lumbar bone density than eumenorrhoeic, sedentary controls - and this was not the case. DPA measures both trabecular and cortical bone with vertebral body trabeculae only contributing 35-50 % of the total (Nottestad et al, 1987). The method of bone density measurement and the small size of the cohort investigated, may explain why this study was unable to detect differences between the menstrual and sporting groups.

The factors that are important in attaining peak bone mass in the mid-thirties are not known. Physical activity tends to increase peak bone mass and might therefore reduce the risk of osteoporosis in later life. However this study confirms that if the intensity of exercise is so high that it leads to amenorrhoea the benefits can be lost.

We have shown that intensive exercise directed at a specific site of the skeleton (i.e. the spine in rowing) may confer additional benefit to bone mineralization at that site. If these positive effects can be preserved into later life, this study suggests that exercise programmes targeted at specific skeletal sites, such as the spine and the femoral neck, may provide valuable clinical strategies for the management of postmenopausal osteoporosis.

6.1.5 CONCLUSIONS

1. Spinal trabecular bone density is markedly reduced in athletes with amenorrhoea ($P = 0.0002$).

2. Spinal trabecular bone density is increased in rowers compared to non-rowers ($P = 0.05$).

3. The exercise performed by rowers may partially compensate the effects of low oestrogen status on trabecular bone density.

4. High levels of exercise in the form of rowing together with normal oestrogen status may lead to increased spinal trabecular bone density.

TABLE 6.1.1
VARIABLES IN THE 10 AMENORRHOEIC LIGHTWEIGHT ROWERS

Reg. No	AGE (yrs)	HEIGHT (cm)	WEIGHT (Kg)	LUMBAR SPINE TBD (mg/cm^3)
006	30	168	60	188
007	27	168	57	216
008	30	166	62	154
014	22	173	62	176
019	22	171	60	191
024	20	174	53	185
027	22	175	61	160
028	21	163	54	200
035	26	168	58	197
061	21	161	63	191

TABLE 6.1.2
VARIABLES IN THE 9 EUMENORRHOEIC LIGHTWEIGHT ROWERS

Reg. No	AGE (yrs)	HEIGHT (cm)	WEIGHT (Kg)	LUMBAR SPINE TBD (mg/cm^3)
011	26	165	59	181
017	27	168	56	210
025	20	171	64	196
032	25	159	55	238
033	24	173	69	199
036	25	171	57	204
020	31	171	63	196
039	27	169	56	283
058	29	169	66	224

TABLE 6.1.3
VARIABLES IN THE 15 AMENORRHOEIC NON-ROWERS.

Reg. No	SPORT	AGE (yrs)	HEIGHT (cm)	WEIGHT (Kg)	LUMBAR SPINE TBD (mg/cm^3)
023	Dancer	20	161	43	168
026	Dancer	22	165	49	115
031	Dancer	18	171	56	121
042	Dancer	21	164	50	144
043	Runner	26	161	51	221
046	Runner	27	164	47	148
052	Runner	25	156	44	149
060	Runner	20	167	49	221
062	Runner	25	170	54	198
063	Runner	27	162	48	96
064	Runner	27	164	47	105
065	Runner	28	163	49	226
066	Runner	22	160	48	115
068	Runner	28	155	46	149
069	Runner	28	153	43	158

TABLE 6.1.4
VARIABLES IN THE 12 EUMENORRHOEIC NON-ROWERS.

Reg. No	SPORT	AGE (yrs)	HEIGHT (cm)	WEIGHT (Kg)	LUMBAR SPINE TBD (mg/cm^3)
037	Dancer	27	165	50	199
038	Dancer	22	162	55	187
040	Dancer	24	167	49	214
048	Runner	29	163	53	178
051	Runner	30	167	51	223
053	Runner	22	160	46	247
054	Runner	23	168	65	210
055	Dancer	30	159	50	206
056	Dancer	20	165	58	180
059	Runner	26	172	61	266
067	Runner	28	170	55	168
070	Runner	25	167	58	197

TABLE 6.1.5 : COMPARISON OF THE SED. CONTROLS AND THE AMENORRHOEIC AND EUMENORRHOEIC LIGHTWEIGHT ROWERS.

	SEDENTARY CONTROLS	AMENORRHOEICS	EUMENORRHOEICS
NUMBER	13	10	9
AGE (Yrs)	28 (4.1)	24 (3.8)	26 (3.1)
HEIGHT (cm)	160 (4.7)	169 (4.6)	168 (4.2)
WEIGHT (kg)	55 (7.1)	59 (3.4)	61 (5.1)
TRABECULAR BONE DENSITY (mg/cm^3)	187 (168,205)	186 (170,202)	215 (199,231)

NB for age, height and weight the figures in brackets are the standard deviations, whereas for trabecular bone density they represent the confidence intervals.

TABLE 6.1.6 : MEAN (S.D.) BACKGROUND VARIABLES FOR ROWERS AND NON-ROWERS

	ROWERS	NON-ROWERS	P-VALUE
NUMBER	19	27	-
Amenorrhoeic	10	15	-
Eumenorrhoic	9	12	-
AGE (Yrs)	25.1 (3.5)	24.9 (3.4)	0.86
WEIGHT (kg)	59.8 (4.2)	51.3 (5.4)	<0.0001
HEIGHT (cm)	169 (4.3)	164 (4.8)	0.001

TABLE 6.1.7 : MEAN (95% CI) SPINAL TRABECULAR BONE DENSITY (mg/cm^3).

	ROWERS	NON-ROWERS	OVERALL
AMENORRHOEICS	186 (170,202)	156 (139,173)	168 (154,181)
EUMENORRHOEICS	215 (199,231)	206 (187,226)	210 (195.225)
OVERALL	199 (184,215)	178 (154,191)	187

TABLE 6.1.8 : MULTIPLE REGRESSION ANALYSIS OF BMD

VARIABLE	STAGES		
	1 $F_{1,44}$	2 $F_{1,43}$	3 $F_{1,42}$
Age	0.98	0.05	0.04
Height	2.18	0.97	0.01
Weight	4.70	1.22	0.13
Sport	3.31	**4.06** (P = 0.05)	
Menstat	**17.08** (P = 0.0002)		
Sport x Menstat (Interaction)			1.20 (P = 0.28)

F-Ratios test whether the entry of each variable would significantly improve the model at each stage of the analysis. The best additional variable at each stage is underlined.

TABLE 6.1.9
BACK STRENGTH MEASUREMENTS IN THE ROWERS

REG. NO	PSOAS X-SECTION AT L3 (mm^2)	PEAK TORQUE FLEX (N.m) 80 $deg.sec^{-1}$	PEAK TORQUE EXT (N.m) 80 $deg.sec^{-1}$
006	857	96	146
007	1078	108	201
008	825	129	157
011	520	126	157
014	1122	159	263
017	1099	161	194
019	948	140	165
020	1084	133	183
024	766	114	279
025	968	137	258
027	865	138	199
028	930	197	129
032	904	115	182
033	938	174	285
035	812	113	220
036	1269	140	285
039	805	136	220
058	847	160	255
061	920	137	282

TABLE 6.1.10
BACK STRENGTH RESULTS IN THE NON-ROWERS

REG. NO	SPORT	PSOAS X-SECTION AT L3 (mm^2)	PEAK TORQUE FLEX (N.m) 80 $deg.sec^{-1}$	PEAK TORQUE EXT (N.m) 80 $deg.sec^{-1}$
023	Dancer	674	100	209
026	Dancer	779	95	156
031	Dancer	777	110	180
037	Dancer	791	123	133
038	Dancer	903	111	168
040	Dancer	1376	96	152
042	Dancer	735	84	163
055	Dancer	753	84	123
056	Dancer	913	127	274
043	Runner	639	127	138
046	Runner	737	85	171
048	Runner	691	108	236
051	Runner	594	110	207
052	Runner	794	107	171
053	Runner	529	113	171
054	Runner	834	137	263
059	Runner	913	115	183
060	Runner	810	103	172
062	Runner	982	103	140
063	Runner	733	*	*
064	Runner	681	76	80
065	Runner	452	92	178
066	Runner	650	85	160
067	Runner	728	167	159
068	Runner	917	95	155
069	Runner	865	92	167
070	Runner	808	111	224

* indicates missing data

TABLE 6.1.11: MEAN (S.D.) BACK STRENGTH MEASUREMENTS

	Non-Rowers	Rowers	P Value
PSOAS CROSS-SECTIONAL AREA AT L3 (mm^2)	780 (171)	928 (167)	0.006
Peak Torque in flexion (N.m)	106 (20)	137 (24)	<0.0001
Peak Torque in Extension(N.m)	174 (42)	214 (52)	0.007

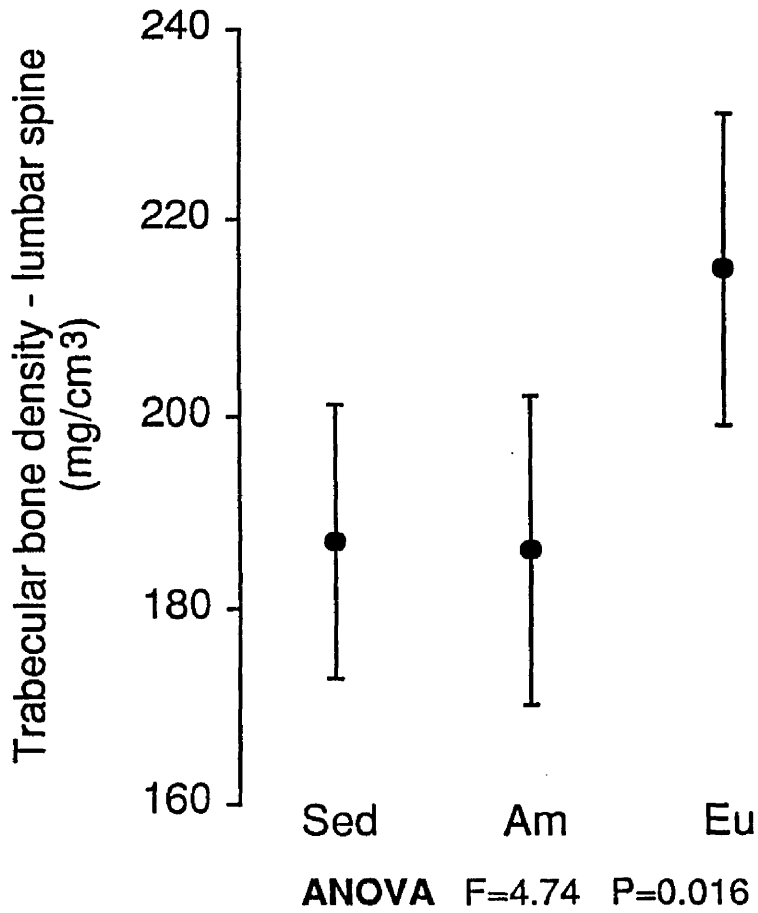

FIGURE 6.1.1

Trabecular Bone Density in the Eumenorrhoeic, Sedentary Controls and the Amenorrhoeic and Eumenorrhoeic Rowers.

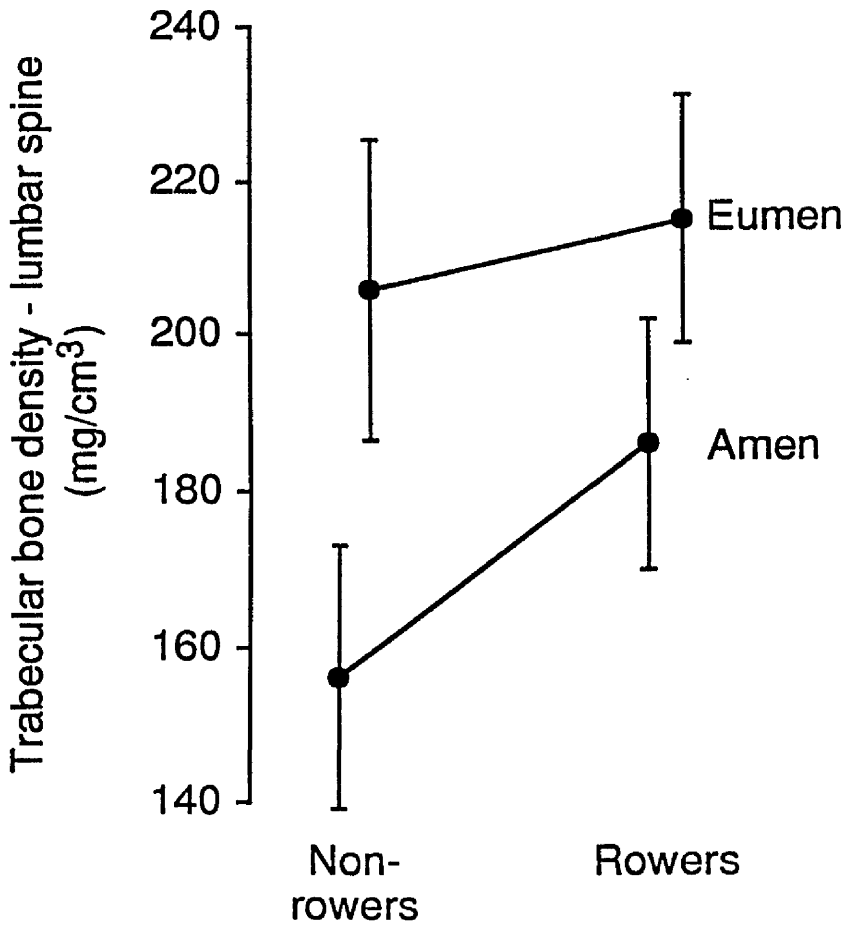

FIGURE 6.1.2

Trabecular Bone Density in the Amenorrhoeic and Eumenorrhoeic Non-Rowers and the Amenorrhoeic and Eumenorrhoeic Rowers.

6.2 EFFECT OF RUNNING ON THE BONE MINERAL CONTENT OF THE FEMORAL MID-SHAFT.

6.2.1 INTRODUCTION

We have already shown that amongst the athletes the bone mineral content of the femoral midshaft is unaffected by menstrual status (chapter 5). To determine the effect of physical training we have investigated the bone mineral content in the femoral midshaft in each of the 3 sporting groups (dancers, rowers and runners) and compared them to the eumenorrhoeic, control group.

Studies in humans have also shown a direct relationship between aerobic capacity ($\dot{V}O_2max$) and BMD (chapter 2.2.2). We have measured $\dot{V}O_2max$ to see if such a relationship exists in elite athletes in whom the aerobic capacity is much higher.

6.2.2 METHOD

The 67 athletes studied consisted of 36 rowers, 21 runners and 10 dancers. Amongst this group were 25 amenorrhoeic, 27 eumenorrhoeic and 15 oral contraceptive-taking athletes (table

3.3 and chapter 3.3). These athletes were compared to a group of 13 eumenorrhoeic, sedentary women of similar age who acted as a control group.

For each subject, height and weight were recorded. The aerobic capacity ($\dot{V}O_2max$) was measured in each athlete using a Tunturi EL 400 cycle ergometer. Continuous expired gas analysis was measured with the Jaeger EOS Sprint automated system and the heart rate (HR) determined with a Rigel Cardiac Monitor (chapter 3.5.3).

Bone mineral content (BMC) of the mid-shaft of the right femur was measured using a Novo BMC-Lab 22a dual photon absorptiometer (chapter 3.4.1).

Daily calcium intake was assessed by questionnaire as described in chapter 3.7.

Statistical Methods

One-way analysis of variance (ANOVA) was used to test whether BMC, and demographic variables such as age, height and weight differed between the four groups (dancers, rowers, runners and sedentary controls). The residuals from the ANOVA were tested for Normality using the Shapiro Wilk's W test (Royston, 1982) and for equal variances in the groups using the Schweder test (Schweder,

1981). When the ANOVA was significant, indicating that there were differences between the groups, the Tukey procedure (Fleiss, 1986) was used to determine where the individual differences lay. Confidence intervals for the means of individual groups were constructed using the degrees of freedom and mean square error from the ANOVA.

In addition linear models (McCullagh and Nelder, 1983; GLIM, 1986) were used to assess which of the 5 variables (sporting group, menstrual status, age, height and weight), or combination of them could best explain the variation in femoral shaft BMC (see "Statistical Methods", chapter 6.1.2)

Analysis of covariance (Snedecor et al, 1980) was also used to see if the relationship between BMC and $\dot{V}O_2max$ varied between the sporting groups.

6.2.3 RESULTS

Tables 6.2.1, 6.2.2 and 6.2.3 give the individual results in the dancers, runners and rowers respectively. Table 5.4 gives the results for the eumenorrhoeic, sedentary controls.

Table 6.2.4 gives details of the demographic variables in the groups. The ANOVA showed that age, weight and height varied significantly between the 4 groups. The Tukey procedure showed

that the sedentary controls were older (28.4 yrs) than the dancers (22.8 yrs) and the rowers (24.7 yrs) with the runners (26.0 yrs) in between. The rowers were both heavier (62.9 Kgs) and taller (171 cm) than the other 3 groups who were similar for these 2 parameters. $\dot{V}O_2$max also varied significantly between the 3 sporting groups being highest in the runners (59.9 mls/Kg/min) who were higher than the rowers (53.8) who, in turn, were higher than the dancers (45.5).

Figure 6.2.1 gives the mean bone mineral content (BMC) and 95% confidence intervals for the 4 groups. The ANOVA showed that BMC varied significantly between the sporting groups (P= 0.0026). The BMC in the runners (1.51 gm/cm^2) was significantly higher than the BMC in the rowers (1.43 gm/cm^2), dancers (1.39 gm/cm^2) and sedentary controls (1.40 gm/cm^2) whose levels were similar.

Figure 5.2 gives the mean BMC and 95% confidence intervals in the 3 menstrual groups. In the amenorrhoeic and eumenorrhoeic athletes the mean was 1.45 gm/cm^2 and in the oral contraceptive takers it was 1.46 gm/cm^2. There results were not significantly different from the sedentary control group (P= 0.38).

Using the linear models, only "sporting group" was related to BMC- as shown in figure 6.2.1. Age, height, weight were not related at all to BMC (P= 0.68, 0.46, 0.73 respectively). Furthermore, although $\dot{V}O_2$max varied between the sporting groups,

being highest in the runners, it too was not related to BMC - this applied collectively and to each sporting group individually.

6.2.4 DISCUSSION

Previous studies (Drinkwater et al, 1984; Parker Jones et al, 1985; Marcus et al, 1985) have shown that cortical BMC in the upper limb is not reduced in amenorrhoeic athletes compared to their eumenorrhoeic counterparts. We have shown that cortical bone in the femoral mid-shaft is also unaffected by low oestrogen status.

Nilsson and Westlin (1971) showed that bone density in the femoral shaft is greater in athletes than non-athletes. They were also able to show differences between sports, being higher in weight-lifters than in runners who, in turn, were higher than swimmers. We have also shown major differences between sports with significantly higher levels in runners than in rowers, dancers and non-athletes.

Using linear models we were able to show that although age, height and weight differed between the groups, they did not contribute to the differences in bone mineral content.

At least 2 studies have shown a positive correlation between bone mineral density and aerobic capacity ($\dot{V}O_2max$). Chow et al (1986) showed a significant correlation between $\dot{V}O_2max$ and total body calcium, measured by neutron activation analysis, in 31 postmenopausal women. Pocock et al (1986) also showed a significant correlation in 38 pre- and 46 postmenopausal women between $\dot{V}O_2max$ and BMD measured at the femoral neck and in the lumbar spine.

The above 2 studies differed from ours in several important ways. Both estimated $\dot{V}O_2max$ from the heart-rate whereas we measured oxygen uptake directly. We have measured aerobic capacity in a younger age-group with values at the top end of the range whereas in the other studies the subjects were older and the range for $\dot{V}O_2max$ was much lower. Furthermore the technique used for measuring bone density and the skeletal site from which the measurements were taken varied in all 3 studies and therefore the results are not directly comparable.

We were unable to show any relationship between cortical BMC and $\dot{V}O_2max$ even though the sporting group with the highest bone density (the runners) also had the highest aerobic capacity. We were also unable to show any relationship between BMC and $\dot{V}O_2max$ in any of the individual sporting groups. This suggests that aerobic training per se does not act as a direct anabolic stimulus on bone.

The runners, who had the highest bone densities, do intense weight-bearing exercise running up to 70 miles/week. This produces intense cyclic loading of the lower body. The dancers also do weight-bearing exercise but much of their work consists of slow movements involving coordination, balance and flexibility with less than 10% involving jumping. The amount of cyclic loading is therefore much less. In the rowers a large part of the training is non-weight-bearing. Although weight training of the lower limbs forms part of the training, the degree of cyclic loading is much less than in running. These results suggest that intense cyclic loading produces an anabolic effect on bone mineralization in excess of moderate loading. However avian studies (Rubin and Lanyon, 1984) have shown that only 36 cycles per day (occupying 72 seconds) were sufficient to saturate the anabolic stimulus and there may therefore be important differences between the human and avian models of cyclic loading.

However this being a cross-sectional study, we cannot be certain that the bone density started at the same level in all 4 groups. It is possible that the greater bone densities seen in the runners may have enabled them to train harder and thus be a cause of their ability to do intense training rather than the result of it.

Previous work has shown that the bone density increases at sites of maximum stress. For example, in tennis players bone density levels in the playing arm are substantially higher than in the non-playing arm (Huddleston et al, 1980; Pirnay et al, 1987),

whereas runners have increased bone density in the calcaneus (Williams et al, 1984). We have shown that running may also produce bone density increases higher up the lower limb, at least as far as the mid-shaft of the femur. If these anabolic effects also occur at the femoral neck then running may provide a useful additional strategy in the prevention of postmenopausal osteoporosis.

6.2.5 CONCLUSIONS

1. Cortical bone mineral density in the femoral mid-shaft is unaffected by changes in oestrogen status amongst elite female athletes.

2. Cortical bone mineral density in the femoral mid-shaft is higher in female runners than in rowers or dancers.

3. Cortical bone mineral density in the femoral mid-shaft is unrelated to the aerobic capacity ($\dot{V}O_2max$) in elite female athletes.

TABLE 6.2.1
VARIABLES IN THE 10 DANCERS
(A= amenorrhoea; E= eumenorrhoea; C= oral contraceptive)

REG NO	MENSTRUAL STATUS	AGE (yrs)	HEIGHT (cm)	WEIGHT (Kg)	$\dot{V}O_2max$ (mls/ Kg/min)	BONE MIN CONTENT (g/cm^2)
023	A	20	161	43	51	1.34
026	A	22	165	49	40	1.27
031	A	18	171	56	44	1.31
037	E	27	165	50	49	1.42
038	E	22	162	55	50	1.38
040	E	24	167	49	48	1.45
042	A	21	164	50	42	1.46
055	E	30	159	50	41	1.35
056	E	20	165	58	46	1.39
057	C	24	161	47	44	1.52

TABLE 6.2.2
VARIABLES IN THE 21 RUNNERS
(A= amenorrhoea; E= eumenorrhoea; C= oral contraceptive)

REG NO	MENSTRUAL STATUS	AGE (yrs)	HEIGHT (cm)	WEIGHT (Kg)	$\dot{V}O_2max$ (mls/ Kg/min)	BONE MIN CONTENT (g/cm^2)
043	A	26	161	51	65	1.62
046	A	27	164	47	59	1.29
047	C	27	164	50	55	1.48
048	E	29	163	53	59	1.41
049	C	24	177	57	63	1.62
050	C	29	157	47	54	1.39
051	E	30	167	51	49	1.68
052	A	25	156	44	71	1.61
053	E	22	160	46	59	1.44
054	E	23	168	65	57	1.55
059	E	26	172	61	54	1.53
060	A	20	167	49	50	1.58
062	A	25	170	54	57	1.63
063	A	27	162	48	66	1.44
064	A	27	164	47	67	1.48
065	A	28	163	49	62	1.45
066	A	22	160	48	62	1.46
067	E	28	170	55	59	1.49
068	A	28	155	46	66	1.48
069	A	28	153	43	66	1.50
070	E	25	167	58	58	1.48

TABLE 6.2.3
VARIABLES IN THE 36 ROWERS.
(A= amenorrhoea; E= eumenorrhoea; C= oral contraceptive)

REG NO	MENSTRUAL STATUS	AGE (yrs)	HEIGHT (cm)	WEIGHT (Kg)	$\dot{V}O_2max$ (mls/ Kg/min)	BONE MIN CONTENT (g/cm^2)
001	E	22	183	78	46	1.39
002	E	23	183	74	53	1.44
003	C	25	165	59	63	1.42
004	C	25	176	70	54	1.31
005	C	23	171	59	59	1.50
006	A	30	168	60	62	1.32
007	A	27	168	57	58	1.49
008	A	30	166	62	53	1.34
009	E	20	174	68	61	1.41
010	C	28	171	60	57	1.44
011	E	26	165	59	57	1.23
012	C	25	175	63	47	1.46
014	A	22	173	62	51	1.20
015	C	31	174	66	56	1.45
016	E	25	179	77	50	1.48
017	E	27	168	56	59	1.41
018	E	26	169	70	54	1.55
019	A	22	171	60	57	1.59
020	E	31	171	63	56	1.59
021	E	25	184	76	45	1.46
022	C	25	167	60	51	1.41
024	A	20	174	53	52	1.29
025	E	20	171	64	49	1.40
027	A	22	175	61	61	1.47
028	A	21	163	54	61	1.45
030	C	21	159	52	61	1.42
032	E	25	159	55	59	1.43
033	E	24	173	69	43	1.50
034	C	25	171	59	54	1.49
035	A	26	168	58	57	1.39
036	E	25	171	57	59	1.31
039	E	27	169	56	54	1.41
044	C	23	182	73	49	1.48
045	C	21	172	65	40	1.52
058	E	29	169	66	41	1.47
061	A	21	161	63	49	1.69

TABLE 6.2.4 : DEMOGRAPHIC VARIABLES (mean with 95% confidence interval) IN THE 4 GROUPS.

	Sedentary Controls	Dancers	Rowers	Runners	ANOVA
Number	13	10	36	21	
Mean Age (95% CI) (Yrs)	28.4 (26.5,30.3)	22.8 (20.7, 25.0)	24.7 (23.5, 25.8)	26.0 (24.5, 27.5)	$F_{3,76}$=6.01 P= 0.0011
Mean Weight (95% CI) (Kg)	54.8 (51.3,59.3)	50.7 (46.7, 54.7)	62.9 (60.8, 65.0)	50.9 (48.1, 53.7)	$F_{3,76}$=20.36 P< 0.0001
Mean Height (95% CI) (cm)	161 (157,164)	164 (160,168)	171 (169,173)	164 (161,166)	$F_{3,76}$=14.94 P< 0.0001
Mean $\dot{V}O_2$ max (95% CI) (ml/kg/ min)	-	45.5 (41.9, 49.1)	53.8 (51.9, 55.7)	59.9 (57.4, 64.4)	$F_{3,64}$=21.98 P< 0.0001

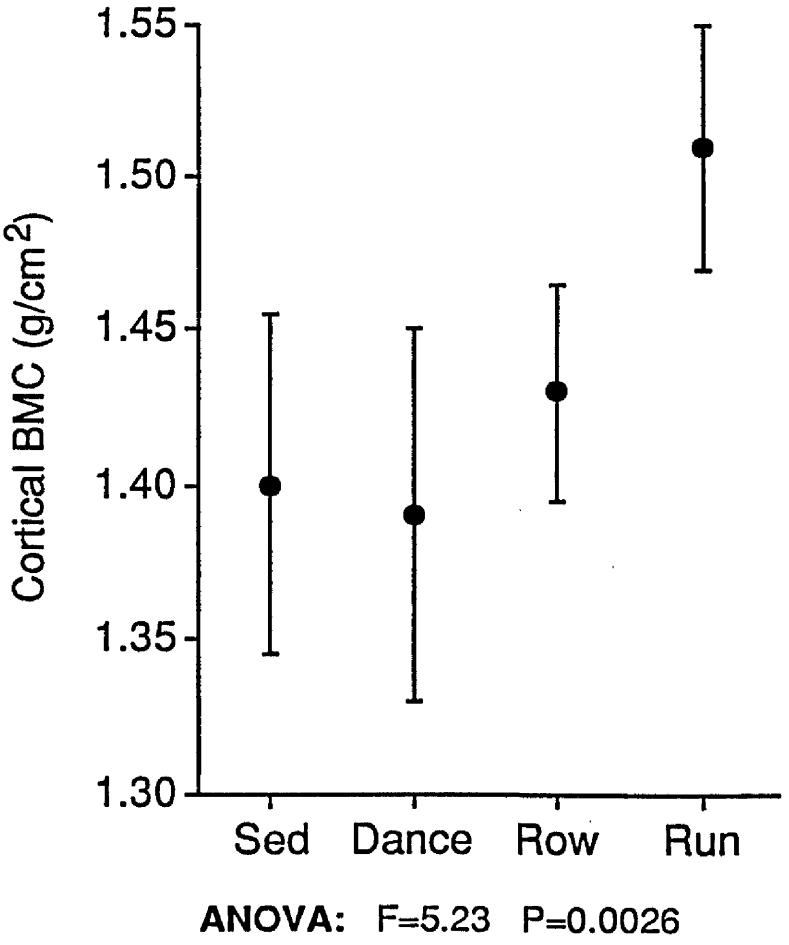

FIGURE 6.2.1

Cortical Bone Mineral Content of Femoral Shaft in Sedentary Controls, Dancers, Rowers and Runners.

CHAPTER 7: RELATIONSHIP BETWEEN FITNESS PARAMETERS AND BONE MINERAL DENSITY.

7.1. INTRODUCTION

Previous work has demonstrated a positive correlation between aerobic capacity ($\dot{V}O_2max$) and bone mineral density in non-athletic populations (chapter 2.2.2). These studies estimated the $\dot{V}O_2max$ from the heart rate.

However Dalsky et al (1988) were unable to show a relationship between aerobic capacity and spinal bone density in a group of postmenopausal women. Bevier et al (1989), using stepwise multiple regression, showed that in elderly women body mass and grip strength were significantly related to spinal bone density but that aerobic capacity and back strength were not.

Studies by Sinaki et al (1986) and by Sinaki and Offord (1988) showed a positive correlation between bone density of the lumbar spine and back extensor strength in 68 postmenopausal women (chapter 2.2.2).

We have investigated the effects of age, height, weight, body composition, isokinetic back strength and aerobic capacity on the bone mineral density of the lumbar spine and the femoral midshaft.

7.2 METHOD

Measurements were performed in all 67 subjects (chapter 3.3)

Aerobic capacity ($\dot{V}O_2max$) was measured directly with exercise performed on a bicycle ergometer (chapter 3.5.3). Back strength was measured isokinetically at a velocity of 80 $deg.sec^{-1}$ (chapter 3.5.4.2). The peak torques during flexion and extension were recorded. Height (cm) and weight (kg) were measured using a Seca 713 stadiometer and platform balance scales. Body fat was estimated using the technique validated by Durnin and Womersley (1974) measuring skinfold thickness at 4 sites (chapter 3.5.2).

Spinal trabecular bone density of the lumbar spine was determined using CT scanning (chapter 3.4.3). Cortical bone density in the femoral midshaft was measured using dual photon absorptiometry (chapter 3.4.1). All the above measurements were performed on the same day.

Statistical Methods

Linear models (McCullagh and Nelder, 1983; GLIM, 1986) were used to assess whether the variation in bone mineral density could best be explained by the differences in sporting activity, menstrual status or by any other background or physiological variable such as age, height, weight, body fat content,

variable such as age, height, weight, body fat content, isokinetic back strength or $\dot{V}O_2max$. The single variable with the greatest significant effect on BMD was incorporated into the model. The remaining variables were assessed in turn and the one with the greatest additional significant effect was included in the model. This procedure was repeated until none of the remaining variables had any significant effect on BMD. The residuals from the final model were tested for Normality using the Shapiro Wilk's W test (Royston, 1982) and for equal variances using the Schweder test (1981).

Analysis of covariance (Snedecor and Cochran, 1980) was also used to see if the relationship between BMD and each variable differed between the sporting or menstrual status groups.

7.3 RESULTS

Tables 5.1, 5.2 and 5.3 give the individual measurements for age, height, weight and bone densities of the lumbar spine and femoral midshaft in the amenorrhoeic, eumenorrhoeic and oral contraceptive-taking athletes respectively.

Tables 7.1, 7.2 and 7.3 give the individual measurements for body fat content, isokinetic back strength and aerobic fitness in the amenorrhoeic, eumenorrhoeic and oral contraceptive-taking athletes respectively. Table 7.4 summarizes these results.

The relationship between spinal trabecular bone density (TBD) and $\dot{V}O_2$max overall is non-linear. It is best represented by a quadratic equation i.e. the TBD is at its lowest at the extremes of $\dot{V}O_2$max and highest in the mid-range. However when this relationship is investigated separately in the low oestrogen status (i.e. the amenorrhoeics) and "normal" oestrogen status (i.e. the eumenorrhoeics and oral contraceptive-takers) groups there are significant differences. Figure 7.1 shows the plotted data and "best fit" line for the amenorrhoeic athletes. This quadratic relationship remained significant (P= 0.015). By contrast, in the "normal" oestrogen status athletes the relationship was linear (P= 0.043) - figure 7.1. However as no woman in this group had a $\dot{V}O_2$max above 63 ml/kg/min it is difficult to know whether the relationship would remain linear above this point.

An inverse relationship exists between $\dot{V}O_2$max and body fat (P <0.001) in both the low and normal oestrogen status athletes respectively (figure 7.2). When body fat was plotted against spinal bone density in the amenorrhoeic athletes the relationship was positive but not significant (P= 0.16) - figure 7.3.

Model fitting was performed to assess which combination of variables exerted the greatest significant effect on lumbar spine bone mineral density. The results are given in table 7.5. Stage 1 shows that menstrual status had the largest effect (P< 0.0001). However the bone mineral density was also related to sport, height, weight, isokinetic back flexion and $\dot{V}O_2$max

(quadratically). When menstrual status was incorporated into the model (Table 7.5, stage 2) the quadratic relationship with $\dot{V}O_2max$ showed the largest effect (P= 0.011), with sport the only other variable to be related significantly to spinal bone density. When menstrual status and the quadratic in $\dot{V}O_2max$ were included in the model (Table 7.5, stage 3) no other variable produced any significant effect.

Model fitting for cortical bone density in the femoral midshaft is shown in table 7.6. The type of sporting activity was the only factor related to bone density at this site (P= 0.003). Once this was incorporated into the model no other variable showed any additional significant effect (table 7.6, stage 2).

7.4 DISCUSSION

We have studied several variables and found that $\dot{V}O_2max$ was the only one to exert an effect on bone mineral density in addition to the sporting activity and menstrual status.

We have demonstrated differing relationships between $\dot{V}O_2max$ and trabecular bone density in the lumbar spine in the 2 oestrogen status groups. In the "normal" oestrogen status athletes there was a positive linear correlation, a feature which has previously been described, while in the "low" oestrogen status group the relationship was non-linear. Trabecular bone density reached a peak in the midrange for $\dot{V}O_2max$ and was at its lowest at the

extremes (figure 7.1). When $\dot{V}O_2$max is high, implying intensive endurance training, an inverse relationship develops and the benefits on spinal bone density are lost. This quadratic relationship has not been reported previously presumably because it has not been studied in subjects in which the $\dot{V}O_2$max is so high. However it is difficult to know whether this is a feature only seen in the amenorrhoeic athlete because no "normal" oestrogen status athlete had a $\dot{V}O_2$max above 63 mls/Kg/min.

We have also shown that $\dot{V}O_2$max is inversely correlated with body fat. In the amenorrhoeic athlete, where ovarian production of oestrogen is severely impaired, the conversion of the adrenal steroid androstenedione to oestrone in peripheral fat cells (Nimrod and Ryan, 1975) may become crucial in maintaining skeletal exposure to oestrogen. The drop in body fat with the rise in $\dot{V}O_2$max will cause a concomitant fall in oestrone production. This fall may reach a critical point where it outweighs the benefits of intense training on the skeleton. This may explain the non-linear relationship between $\dot{V}O_2$max and TBD. However in "normal" menstrual status athletes where the supply of oestrogen is plentiful, the amount produced by peripheral fat cells may not be as crucial and so the benefits of intense exercise on the skeleton are not compromised. In keeping with this hypothesis Linnell et al (1984) found that only amongst the amenorrhoeic athletes was there a positive correlation between bone mineral density and body fat. However they did not go on to investigate the effect of $\dot{V}O_2$max on bone mineral density.

Other studies have investigated the relationship between aerobic fitness and bone mineral density. Pocock et al (1986) estimated $\dot{V}O_2max$ from the heart rate in a large group of women (pre- and post-menopausal) and showed a positive correlation with the BMD at the femoral neck and the lumbar spine. Chow et al (1986) also estimated the $\dot{V}O_2max$ from the heart rate in a group of postmenopausal women and showed a positive correlation with total body calcium. Bevier (1989) measured $\dot{V}O_2max$ directly and showed no correlation with spinal bone density in postmenopausal women. Dalsky (1988) was also unable to show a relationship between $\dot{V}O_2max$ and spinal bone density in group of postmenopausal women, half of whom went on an aerobic training programme during the study. The latter 2 studies measured $\dot{V}O_2max$ directly by gas analysis which is a more accurate measure especially in the elderly where there is evidence to suggest that estimates based on heart rate may be unreliable (Bevier et al, 1989). Furthermore the study by Pocock expressed $\dot{V}O_2max$ in l/min which, in addition to being a measure of aerobic capacity, is also a reflection of body mass which is known to influence bone density.

In contrast to spinal trabecular bone, cortical bone density in the femoral midshaft was not related to $\dot{V}O_2max$ or menstrual status. It is however strongly related to the type of sporting activity, being greatest in extreme weight-bearing exercise (chapter 6.2). Cortical bone responds to changes in oestrogen status in a different way from trabecular bone. Whether cortical

bone also responds to exercise differently, cannot be determined from this study as we were measuring cortical and trabecular bone at different anatomical sites.

We have also been unable to show any relationship between isokinetic back strength and the bone density either in the lumbar spine or femoral midshaft. Sinaki et al (1986) found a significant, positive correlation between spinal bone density and isometric back extensor strength in 68 postmenopausal women. However several other factors were strongly correlated to bone density, in particular height and age. Once these 2 variables were incorporated into a multiple regression model to predict bone density no other variable, including back strength, added any significant effect. Bevier et al (1989) also measured isometric back extensor strength in a group of postmenopausal women and found no relationship with spinal bone density. The group studied by Sinaki was younger than the one reported by Bevier (about 55 years v 70 years) and so they are not directly comparable. As far as we are aware there are no studies linking isokinetic back strength with spinal bone density. We have already shown that menstrual status exerts a substantial effect on TBD in the spine (chapter 5) and this may obscure any minor effect produced by back strength in elite female athletes.

In contrast to previous studies which have concentrated on older, less fit populations, we have been unable to show any strong positive correlations between fitness parameters and bone density. In fact in amenorrhoeic athletes we have shown an

inverse relationship with $\dot{V}O_2max$ and this may suggest that in certain circumstances intense exercise may have a detrimental effect on bone density.

7.5 CONCLUSIONS

1. The Bone Mineral Density in the lumbar spine is influenced by menstrual status, aerobic capacity and by sport whereas in the femoral midshaft only sport exerts an influence.

2. There was an inverse relationship between $\dot{V}O_2max$ and body fat.

3. In the "normal" oestrogen status athletes there was a positive correlation between $\dot{V}O_2max$ and spinal bone density.

4. In the amenorrhoeic athletes there was a non-linear relationship between $\dot{V}O_2max$ and spinal bone density which may be associated with the low levels of body fat at high levels of $\dot{V}O_2max$.

5. Different anatomical sites in the skeleton respond to different types of exercise activity.

6. Cortical and trabecular bone may respond differently to the effects of exercise.

TABLE 7.1
PHYSIOLOGICAL PARAMETERS IN THE 25 AMENORRHOEIC ATHLETES.
(D= dancers; Row= rowers; Ru= runners)

REG.NO	SPORT	BODY FAT(%)	ISOKINETIC BACK STRENGTH PEAK TORQUE (80 deg.sec^{-1}) FLEX (Nm)	EXTN (Nm)	$\dot{V}O_2$max (mls/ Kg/min)
006	Row	22.1	96	146	62
007	Row	19.1	108	201	58
008	Row	25.1	129	157	53
014	Row	21.2	159	263	51
019	Row	14.8	140	165	57
023	D	15.5	100	209	51
024	Row	20.3	114	279	52
026	D	17.4	95	156	40
027	Row	16.1	138	199	61
028	Row	14.6	197	129	61
031	D	22.1	110	180	44
035	Row	22.4	113	220	57
042	D	20.5	84	163	42
043	Ru	17.9	127	138	65
046	Ru	15.2	85	171	59
052	Ru	12.8	107	171	71
060	Ru	16.3	103	172	50
061	Row	27.2	137	282	49
062	Ru	17.9	103	140	57
063	Ru	13.5	*	*	66
064	Ru	11.2	76	80	67
065	Ru	20.7	92	178	62
066	Ru	19.4	85	160	62
068	Ru	13.8	95	155	66
069	Ru	13.6	92	167	66

* - indicates missing data.

TABLE 7.3
PHYSIOLOGICAL PARAMETERS IN THE 15 ORAL CONTRACEPTIVE-TAKERS
(D= dancers; Row= rowers; Ru= runners)

REG.NO	SPORT	BODY FAT(%)	ISOKINETIC BACK STRENGTH PEAK TORQUE (80 deg.sec^{-1}) FLEX (Nm)	EXTN (Nm)	$\dot{V}O_2$max (mls/ Kg/min)
003	Row	18.8	104	176	63
004	Row	21.5	171	279	54
005	Row	14.5	134	174	59
010	Row	19.1	152	122	57
012	Row	20.4	136	271	47
015	Row	18.1	163	263	56
022	Row	25.1	95	194	51
030	Row	16.8	115	236	61
034	Row	22.3	132	179	54
044	Row	17.3	213	306	49
045	Row	23.1	137	201	40
047	Ru	21.4	110	233	55
049	Ru	17.9	140	194	63
050	Ru	18.6	107	145	54
057	D	16.4	94	176	44

TABLE 7.2
PHYSIOLOGICAL PARAMETERS IN THE 27 EUMENORRHOEIC ATHLETES.
(D= dancers; Row= rowers; Ru= runners)

REG. NO	SPORT	BODY FAT(%)	ISOKINETIC BACK STRENGTH PEAK TORQUE (80 deg.sec^{-1}) FLEX (Nm)	EXTN (Nm)	$\dot{V}O_2max$ (mls/ Kg/min)
001	Row	20.4	186	132	46
002	Row	20.4	163	153	53
009	Row	22.8	152	277	61
011	Row	20.1	126	157	57
016	Row	22.6	174	348	50
017	Row	15.8	161	194	59
018	Row	23.5	163	259	54
020	Row	15.8	133	183	56
021	Row	19.5	184	263	45
025	Row	27.6	137	258	49
032	Row	18.4	115	182	59
033	Row	29.4	174	285	43
036	Row	17.9	140	285	59
037	D	18.9	123	133	49
038	D	23.4	111	168	50
039	Row	20.5	136	220	54
040	D	17.7	96	152	48
048	Ru	19.7	108	236	59
051	Ru	17.1	110	207	49
053	Ru	18.1	113	171	59
054	Ru	23.5	137	263	57
055	D	26.1	84	123	41
056	D	20.5	127	274	46
058	Row	20.9	160	255	41
059	Ru	21.5	115	183	54
067	Ru	16	167	159	59
070	Ru	17	111	224	58

TABLE 7.4 : MEAN (SD) DEMOGRAPHIC AND PHYSIOLOGICAL VARIABLES IN THE 3 MENSTRUAL GROUPS

	AMENORRHOEICS	EUMENORRHOEICS	ORAL CONTRACEPTIVE TAKERS
NUMBER	25	27	15
AGE (Yrs)	24.2 (3.5)	25.4 (3.1)	25.1 (2.9)
HEIGHT (cm)	165 (6)	169 (7)	169 (7)
WEIGHT (kg)	53 (6)	61 (9)	59 (8)
BODY FAT (%)	18.0 (4.1)	20.6 (3.5)	19.4 (2.8)
ISOKINETIC BACK STRENGTH			
Flexion (Nm)	112 (28)	137 (28)	133 (32)
Extension (Nm)	178 (47)	213 (59)	210 (53)
$\dot{V}O_2$ **max** (mls/kg/min)	57 (8)	52 (6)	54 (7)

TABLE 7.5: MODEL FITTING FOR TRABECULAR BONE DENSITY (Lumbar Spine)

Variable	Stage 1 P	Stage 2 P	Stage 3 P
Sport	0.009	0.032	0.137
Men. Stat	**< 0.0001**	-	-
Age	0.069	0.788	0.550
Height	0.011	0.170	0.251
Weight	0.005	0.228	0.283
Body Fat	0.164	0.785	0.593
Isokinetic Back Strength (Flexion)	0.008	0.176	0.266
Isokinetic Back Strength (Extn.)	0.082	0.534	0.605
$\dot{V}O_2max$	0.920	0.182	-
$\dot{V}O_2max + \dot{V}O_2max^2$	0.002	**0.011**	-

Final Model: Men.Stat + ($\dot{V}O_2max + \dot{V}O_2max^2$)

Stage 1:
Values in the column represent the probability from the significance test of the relationship between each single variable and the trabecular bone density. The one with the smallest P-value is incorporated into the model.

Stages 2,3:
Values in the columns represent the probabilities from the significance tests of any additional relationship between each remaining variable and the trabecular bone density. The one with the smallest P-value (providing it is < 0.05) is added to the model.

TABLE 7.6 : MODEL FITTING FOR BONE MINERAL CONTENT (FEMORAL MID-SHAFT)

Variable	Stage 1 P	Stage 2 P
Sport	**0.003**	-
Men. Stat	0.902	0.628
Age	0.475	0.716
Height	0.794	0.505
Weight	0.958	0.117
Body Fat	0.218	0.890
Isokinetic Back Strength (Flexion)	0.729	0.167
Isokinetic Back Strength (Extn.)	0.800	0.774
$\dot{V}O_2$max	0.204	0.281

Final Model: Sport

Stage 1:
Values in the column represent the probability from the significance test of the relationship between each single variable and the bone density of the femoral shaft. The one with the smallest P-value is incorporated into the model.

Stage 2:
Values in the column represent the probability from the significance test of any additional relationship between each remaining variable and the bone density of the femoral shaft.

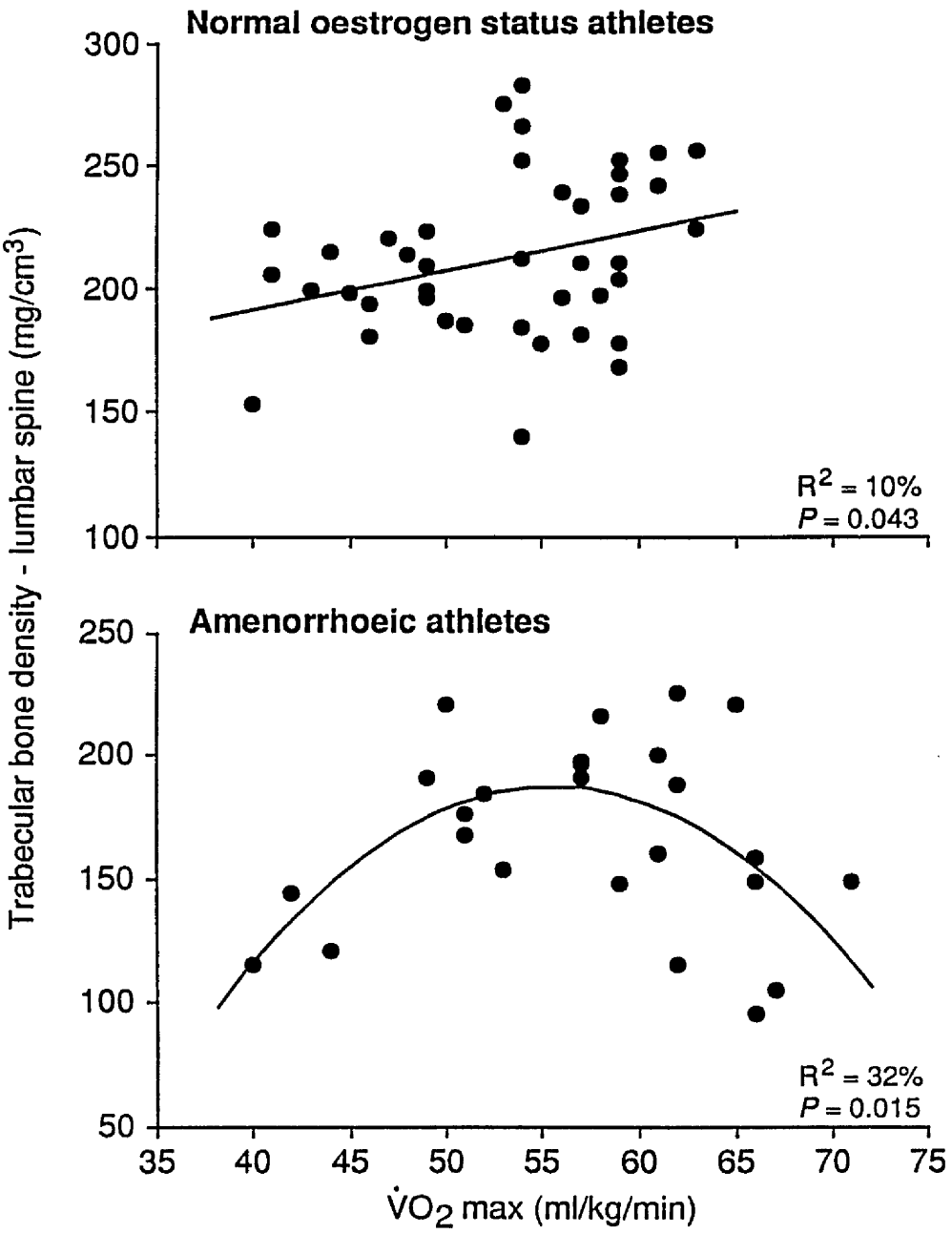

FIGURE 7.1:

Trabecular Bone Density plotted against $\dot{V}O_2$max ("best fit" line) for the Amenorrhoeic and "Normal" Oestrogen Status Athletes.

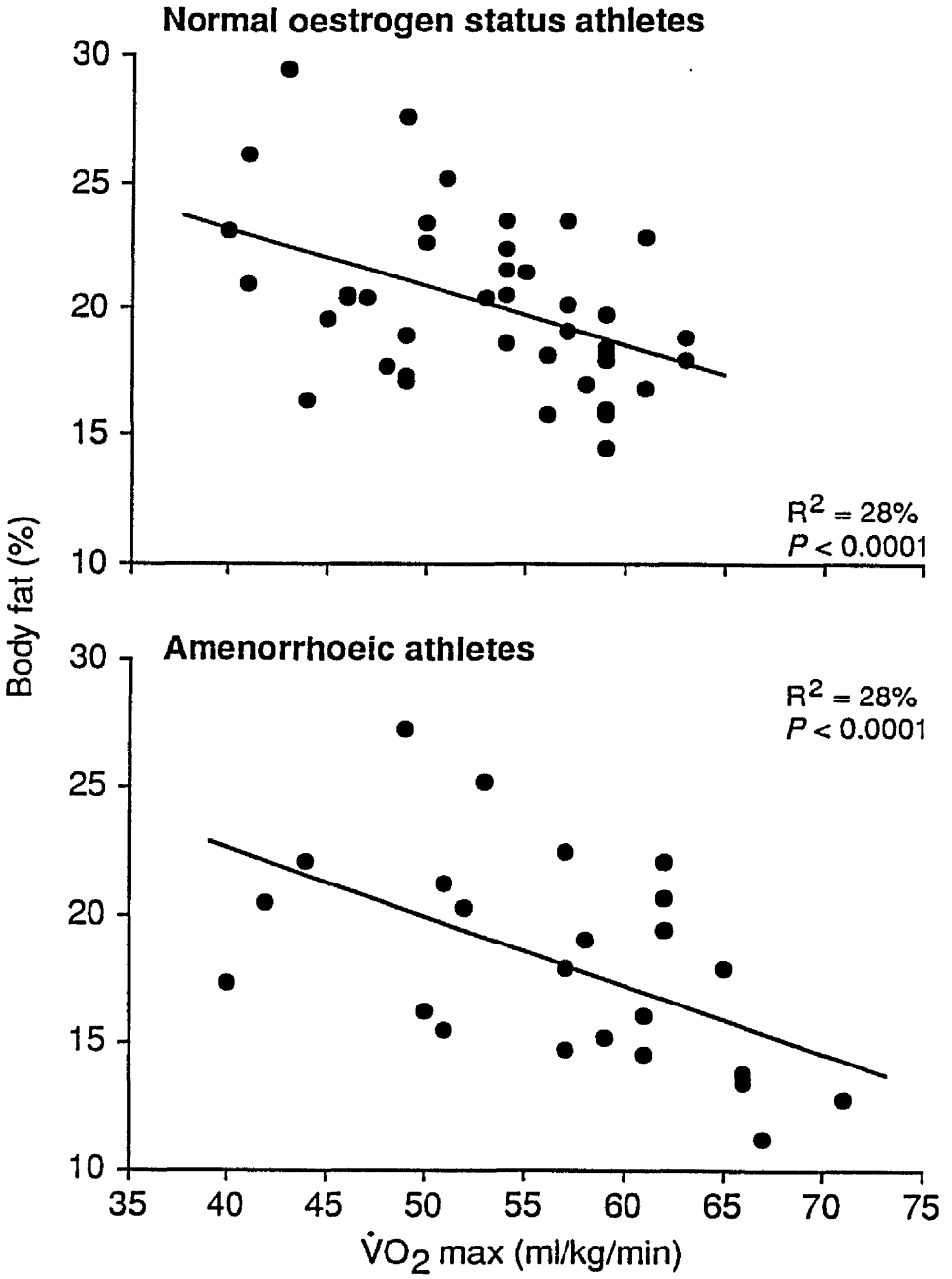

FIGURE 7.2:

Body Fat (%) plotted against $\dot{V}O_2$max ("best fit" line) for the Amenorrhoeic and "Normal" Oestrogen Status Athletes.

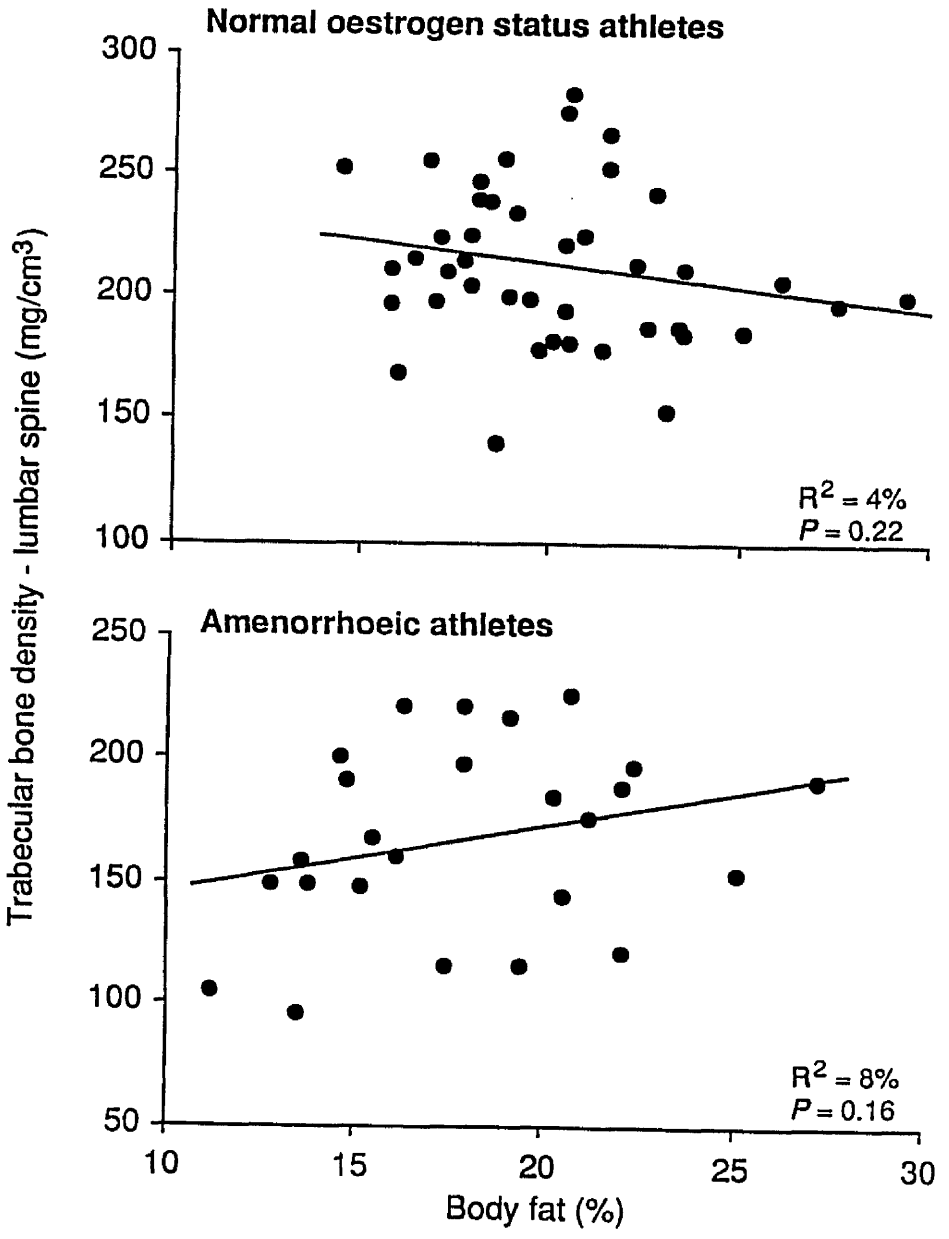

FIGURE 7.3:

Trabecular Bone Density plotted against Body Fat ("best fit" line) for the Amenorrhoeic and "Normal" Oestrogen Status Athletes.

CHAPTER 8: EFFECT OF CALCIUM INTAKE ON BONE MINERAL DENSITY

8.1 INTRODUCTION

Controversy exists concerning the effect of dietary calcium on bone mineral density and fracture risk. Some studies have suggested a weak relationship between calcium intake and bone mineral density while others have shown none (chapter 2.2.3). Work by Heaney, Recker et al, (1978) suggested that, in order to maintain calcium balance, daily calcium intake had to be at least 1000 mg for premenopausal and oestrogen-treated postmenopausal women and 1500 mg for oestrogen-deficient postmenopausal women.

We have measured the calcium intake and investigated its relationship with bone mineral density in the amenorrhoeic and "normal" oestrogen status athletes.

8.2 METHOD

The cohort consisted of the 67 athletes (chapter 3.3) including 25 amenorrhoeic and 42 "normal" oestrogen status (27 eumenorrhoeics and 15 oral contraceptive-takers) athletes (table 3.3). This cohort contained 10 dancers, 36 rowers and 21 runners.

Daily calcium intake (in mg) was measured by questionnaire which was based on the method described by Nelson, Hague et al (1988) - see chapter 3.7.

The cortical bone mineral content of the right femoral midshaft was measured using dual photon absorptiometry (chapter 3.4.1). Trabecular bone density of the lumbar spine was measured using CT scanning (chapter 3.4.3).

Statistical Methods

The effects of daily calcium intake, menstrual status and sporting activity group on bone mineral density were investigated. Linear models (McCullagh and Nelder, 1983; GLIM, 1986) were used to assess which of these 3 variables, or combination of them, could best explain the variation in bone mineral density at each of the 2 sites (see "Statistical Methods", chapter 7.2).

8.3 RESULTS

The daily calcium intake and the bone density measurements for each amenorrhoeic and "normal" oestrogen status athlete are given in tables 8.1 and 8.2 respectively.

The mean calcium intake in the amenorrhoeics and "normal" oestrogen status athletes was similar (778 mg and 702 mg respectively). The mean age was also similar in the 2 groups (24.2 and 25.3 yrs respectively).

Table 8.3 gives the results of model fitting for trabecular bone density in the spine. As shown in chapter 7, menstrual status exerts the greatest significant effect on trabecular bone density ($P<0.001$). Once this is incorporated into the model, daily calcium intake exerts the biggest additional significant effect ($P= 0.002$) and when these 2 variables are incorporated (table 8.4, stage 3) sporting activity adds no further significant effect.

The relationship between trabecular bone density (TBD) and daily calcium intake is linear. There was no significant interaction between the effects of menstrual status and calcium intake on TBD ($P= 0.72$) suggesting that the relationship between calcium intake and bone density applies equally to both menstrual states i.e. the regression lines are parallel. This is illustrated in figure 8.1 and shows that TBD increases with calcium intake in both groups, but is significantly lower in the amenorrhoeics at all levels of calcium intake. The regression lines can be represented by the following equations:-

1) For low oestrogen status athletes $Y = 0.039X + 137.1$

2) For normal oestrogen status athletes $Y = 0.039X + 184.5$

where X = daily calcium intake and Y = trabecular bone density.

From these equations it can be seen that for every 100 mg increase in daily calcium intake, the trabecular bone density rises by 3.9 mg cm^{-3}. Furthermore, assuming that the lines remain linear at higher levels of calcium intake and that there is a direct causal relationship, it would require an increase in daily calcium intake of 1203 mg to shift the trabecular bone density from the low to normal oestrogen status line.

Daily calcium intake was unrelated to the bone mineral content of the femoral midshaft. Sporting activity was the only variable related to bone density at this site (P= 0.003), a relationship demonstrated in chapter 7 (table 7.6).

8.4 DISCUSSION

A recent review by Kanis and Passmore (1989) concluded that calcium supplementation to the diet was unnecessary, but this was based primarily on data from women in the peri- and post-menopausal period. There is little information available on the effect in childhood and young adults.

Sandler et al (1985) showed a positive relationship between calcium consumption in childhood and adolescence and bone density in the postmenopausal period. This study was based on dietary recall from 40 years previously and hence should be interpreted

with caution. Kanders et al (1988) investigated a group of 60 eumenorrhoeic women between the ages of 25 and 34 and showed a positive linear correlation between calcium intake and vertebral bone density. This was only present for daily calcium intakes up to 1000 mg, with no relationship with intakes above this level.

We have shown a positive, linear correlation between daily calcium intake and spinal trabecular bone density in a group of young women in their twenties. This applies equally to the low and "normal" oestrogen status athletes. However although the model fittings performed in chapters 7.3 and 8.3 suggest that dietary calcium intake exerts a stronger influence on spinal bone density than either $\dot{V}O_2max$ or Sporting activity group, it does not necessarily imply that there is a direct causal link between dietary calcium and spinal bone density. There may be one or several intermediate factors that both are related to. For example, calcium intake is closely related to energy intake (chapter 2.2.3.3) which in turn is related to energy expenditure and activity and it may be this later factor that is directly related to spinal bone density. To determine the effect of dietary calcium on spinal bone density, we would need to control for calorie intake, a feature which was not included in the design of the study. This is discussed further in chapter 11.

For any given calcium intake the bone density was significantly lower in the amenorrhoeic athletes. If there is a causal relationship between calcium intake and bone density and it remains linear at higher intakes, the low oestrogen status women

would require an extra 1200 mg of calcium daily to reach the trabecular bone density of their normal oestrogen status counterparts. Although Kanders et al (1988) showed no benefit to bone density with intakes above 1000 mg, calcium absorption studies performed by Heaney et al (1975) in normal adults showed that as intake increases, the rate of absorption decreases but the absolute amount of calcium absorbed continues to rise. This study included intakes up to 7000 mg per day. However it did not investigate the relationship between calcium absorption and bone density.

Studies on oestrogen-deficient women after the menopause have shown that oestrogen replacement is far more effective at preserving bone mineral density than calcium supplementation (chapter 2.2.3) but that the latter may also have some beneficial effect. Calcium balance studies by Heaney, Recker et al (1978) suggested that oestrogen-deficient postmenopausal women required 1500 mg of calcium per day which was 500 mg more than the requirements of those receiving oestrogen replacement.

These studies suggest that calcium requirement is higher in women who are oestrogen-deficient. Calcium absorption from the gut is impaired in oestrogen deficiency (Civitelli et al, 1988), but whether this is the cause or the result of bone mineral loss remains uncertain (Kanis and Passmore, 1989). It has been suggested that oestrogen, in addition to enhancing tubular reabsorption of calcium, stimulates the production of 1,25 dihydroxyvitamin D; in oestrogen-deficient women there is a

resultant rise in urinary calcium loss and fall in 1,25 dihydroxyvitamin D which leads to impaired calcium absorption from the gut. Increased bone resorption might then result in release of calcium into the plasma to offset the calcium losses elsewhere.

The alternative hypothesis is that oestrogen has a direct action on bone which is supported by the fact that oestrogen receptors have recently been discovered at this site (see chapter 2.4.1.4). Oestrogen deficiency leads to an increase in bone resorption with consequent calcium infusion into the plasma. Reduced intestinal absorption would then result as a homeostatic response to hypercalcaemia and thus be the result of bone mineral loss. Until this dilemma is resolved, the value of calcium supplementation in oestrogen deficiency will remain uncertain (Kanis and Passmore, 1989; Nordin and Heaney, 1990).

Our study suggests that increasing dietary calcium in the twenties may enhance peak bone mass (see chapter 11) which theoretically could reduce the risk of osteoporosis in later life.

8.5 CONCLUSIONS

1. Daily calcium intake is positively correlated with spinal trabecular bone density in female athletes in their twenties.

2. The relationship applies equally to the low and normal oestrogen status athletes.

3. In order to increase the trabecular bone density to the level of the normal oestrogen status athletes and assuming a direct causal link, the amenorrhoeics would, on average, have to increase their daily calcium intake by about 1200 mg.

4. Daily calcium intake is not related to cortical bone density in the femoral midshaft.

TABLE 8.1

DAILY CALCIUM INTAKE AND BONE DENSITY MEASUREMENTS IN THE 25 AMENORRHOEIC ATHLETES.
(D= dancers; Row= rowers; Ru= runners)

Reg. No.	SPORT	AGE (yrs)	LUMBAR SPINE TBD (mg/cm^3)	FEM.SHAFT BMC (gm/cm^2)	DAILY CALCIUM INTAKE (mg)
006	Row	30	188	1.32	1097
007	Row	27	216	1.49	1376
008	Row	30	154	1.34	440
014	Row	22	176	1.20	932
019	Row	22	191	1.59	248
023	D	20	168	1.34	975
024	Row	20	185	1.29	723
026	D	22	115	1.27	279
027	Row	22	160	1.47	590
028	Row	21	200	1.45	855
031	D	18	121	1.31	578
035	Row	26	197	1.39	1540
042	D	21	144	1.46	801
043	Ru	26	221	1.62	405
046	Ru	27	148	1.29	863
052	Ru	25	149	1.61	536
060	Ru	20	221	1.58	551
061	Row	21	191	1.69	1002
062	Ru	25	198	1.63	1240
063	Ru	27	96	1.44	580
064	Ru	27	105	1.48	551
065	Ru	28	226	1.45	808
066	Ru	22	115	1.46	452
068	Ru	28	149	1.48	1022
069	Ru	28	158	1.50	1007

TABLE 8.2

DAILY CALCIUM INTAKE AND BONE DENSITY MEASUREMENTS IN THE 42 NORMAL OESTROGEN STATUS ATHLETES.
(D= dancers; Row= rowers; Ru= runners)

Reg. No.	SPORT	AGE (yrs)	LUMBAR SPINE TBD (mg/cm^3)	FEM.SHAFT BMC (gm/cm^2)	DAILY CALCIUM INTAKE (mg)
001	Row	22	193	1.39	590
002	Row	23	276	1.44	1724
009	Row	20	242	1.41	989
011	Row	26	181	1.23	225
016	Row	25	187	1.48	893
017	Row	27	210	1.41	710
018	Row	26	184	1.55	833
020	Row	31	196	1.59	554
021	Row	25	198	1.46	1002
025	Row	20	196	1.40	375
032	Row	25	238	1.43	872
033	Row	24	199	1.50	500
036	Row	25	204	1.31	218
037	D	27	199	1.42	699
038	D	22	187	1.38	607
039	Row	27	283	1.41	957
040	D	24	214	1.45	497
048	Ru	29	178	1.41	892
051	Ru	30	223	1.68	130
053	Ru	22	247	1.44	621
054	Ru	23	210	1.55	725
055	D	30	206	1.35	490
056	D	20	180	1.39	688
058	Row	29	224	1.47	502
059	Ru	26	266	1.53	506
067	Ru	28	168	1.49	1054
070	Ru	25	197	1.48	708
003	Row	25	256	1.42	601
004	Row	25	252	1.31	1586
005	Row	23	252	1.50	706
010	Row	28	234	1.44	1110
012	Row	25	221	1.46	481
015	Row	31	239	1.45	612
022	Row	25	185	1.41	384
030	Row	21	235	1.42	813
034	Row	25	212	1.49	644
044	Row	23	209	1.48	983
045	Row	21	153	1.52	865
047	Ru	27	178	1.48	454
049	Ru	24	224	1.62	640
050	Ru	29	140	1.39	313
057	D	24	215	1.52	732

TABLE 8.3 : Model Fitting for Trabecular Bone Density (Lumbar Spine)

VARIABLE	Stage 1 P	Stage 2 P	Stage 3 P
Menstrual Status	**<0.0001**	-	-
Calcium Intake	0.043	**0.002**	-
Sport	0.009	0.032	0.10
Interaction Between Men. Stat & Calcium Intake	-	-	0.72

Stage 1:
Values in the column represent the probability from the significance test of the relationship between each single variable and the trabecular bone density. The one with the smallest P-value is incorporated into the model.

Stage 2,3:
Values in the columns represent the probabilities from the significance tests of any additional relationship between each remaining variable and the trabecular bone density. The one with the smallest P-value (providing it is < 0.05) is added to the model.

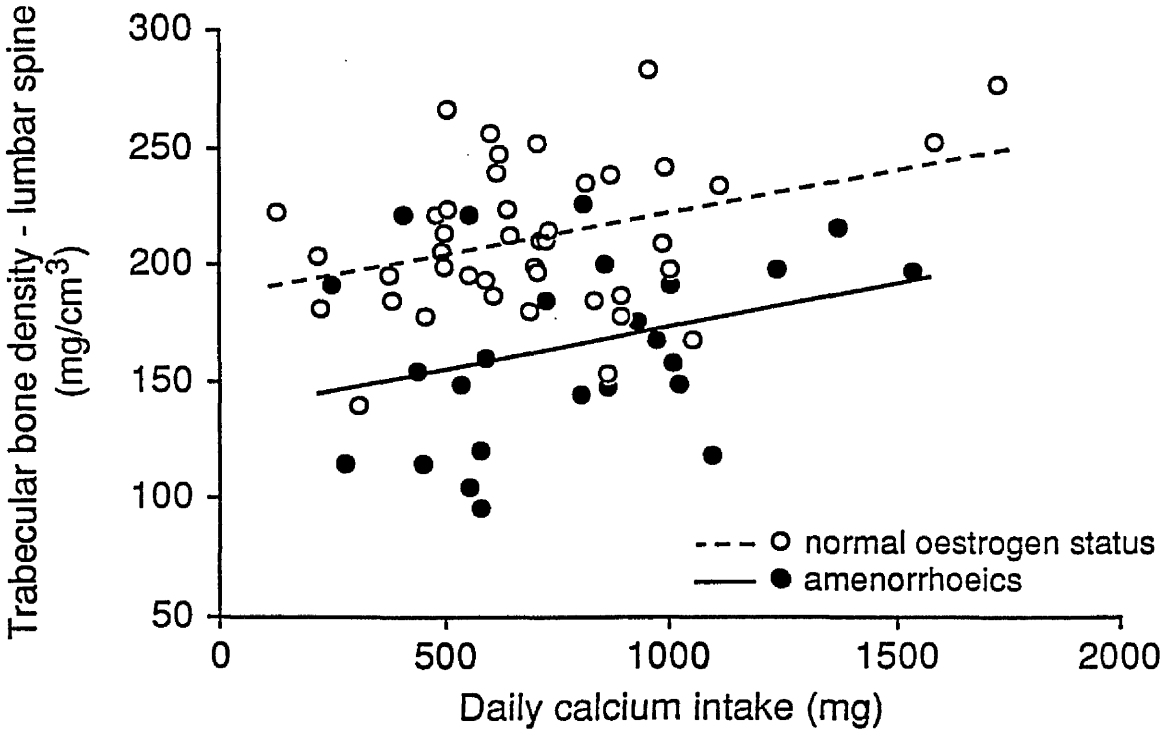

Figure 8.1:

Relationship between daily calcium intake and trabecular bone density in the amenorrhoeic and normal oestrogen status athletes ((P=0.0023).

CHAPTER 9: RELATIONSHIP BETWEEN OESTROGEN STATUS AND BONE BIOCHEMISTRY

9.1 INTRODUCTION

Although oestrogen is known to exert an important effect on bone metabolism, the mechanism of its action remains uncertain. Recent studies have shown oestrogen receptors on osteoblasts (chapter 2.4) while others have found changes in Calcitonin, PTH and Vitamin D in response to changes in oestrogen status. Oestrogen is thought to act by inhibiting bone resorption with little effect on formation.

Most work has concentrated on the postmenopausal period. We have investigated the effect of altered oestrogen status on bone metabolism amongst young female athletes.

9.2 METHOD

There were 67 athletes in the study; 25 with amenorrhoea (amen), 27 with eumenorrhoea (eumen) and 15 on the oestrogen-containing oral contraceptive (OC)- chapter 3.3, table 3.3.

A venous sample was taken from each subject between 9.00 and 10.00 am. Serum calcium, phosphate, albumin and alkaline phosphatase were measured using a multi-channel analyzer (chapter 3.6.1). Serum osteocalcin (active fraction and total amount) was measured by radioimmunoassay (chapter 3.6.5). Serum 25-hydroxyvitamin D was measured using a competitive protein-binding technique (chapter 3.6.3) and parathyroid hormone by radioimmunoassay (chapter 3.6.4).

Each subject provided urine from the second sample of the day, having been on a gelatin-free diet for the previous 24 hours (chapter 3.6.2). Calcium, hydroxyproline and creatinine were measured by the methods described in chapter 3.6.2.

Statistical Methods

The difference in the biochemical parameters between the 3 menstrual groups was assessed by analysis of variance. Pearson product moment correlation coefficients were used to investigate the relationship between the biochemical parameters and between the biochemical parameters and $\dot{V}O_2max$ (chapter 3.5.3), isokinetic back strength (chapter 3.5.4) and body fat (chapter 3.5.2).

9.3 RESULTS

Tables 9.1 and 9.4 give the individual biochemical measurements for the amenorrhoeic athletes, 9.2 and 9.5 for the eumenorrhoeic athletes and 9.3 and 9.6 for the oral contraceptive takers. Amongst the eumenorrhoeic athletes two (study numbers 020 and 039) were unable to provide sufficient urine to measure the hydroxyproline:creatinine ratio. One eumenorrhoeic athlete (055) had a stress fracture of the metatarsus with an associated high alkaline phosphatase of 476 IU/l. As this abnormal value was associated with a well defined cause, it was excluded from the statistical analysis. Table 9.7 summarizes this data, giving the mean (and 95% confidence interval) values for all the variables in each of the 3 menstrual groups. The F ratio and P values from the analysis of variance are also given.

Alkaline Phosphatase (figure 9.1) was significantly higher (P= 0.04) in the Amen (72 IU/l) than in the OC athletes (59 IU/l) with the Eumen athletes in between (66 IU/l). Total osteocalcin was significantly higher (P= 0.006) in the Eumen (3.49 ng/ml) than in the OC group (2.47 ng/ml) with the Amen group (3.06 ng/ml) in between (figure 9.2). Active osteocalcin showed similar differences between the menstrual states.

The ratio of active to total osteocalcin was similar in all 3 groups being 83% in the amenorrhoeics, 81% in the eumenorrhoeics and 77% in athletes on the oral contraceptive.

The urinary calcium:creatinine ratio also varied between the 3 groups (P= 0.011) being higher in the Amen (0.46) than in the OC athletes (0.24) with the Eumen athletes (0.34) in between (figure 9.3). There was no significant difference in the urinary hydroxyproline:creatinine ratio between the 3 groups (figure 9.4).

Parathyroid hormone, 25-hydroxyvitamin D, calcium, phosphate and albumin did not vary between the 3 groups.

The relationship between the parameters of bone turnover, parathyroid hormone and 25-hydroxyvitamin D are given in table 9.8. Alkaline phosphatase and total osteocalcin (both measures of bone formation) were significantly correlated (r= 0.313; P= 0.01). PTH and 25-hydroxyvitamin D showed a significant inverse correlation (r= -0.265; P= 0.03) but there were no other significant relationships.

The individual measurements for $\dot{V}O_2max$, isokinetic back strength and body fat in the amenorrhoeic, eumenorrhoeic and oral contraceptive-taking athletes are given in tables 7.1, 7.2 and 7.3 respectively.

The relationship between the biochemical and exercise parameters ($\dot{V}O_2max$, isokinetic back flexion and extension, and body fat) are given in table 9.9. The only significant correlations present

were between urinary calcium:creatinine and $\dot{V}O_2max$ (r= 0.284; P= 0.02) and between osteocalcin and isokinetic back extension (r= 0.275; P= 0.03).

As we were performing multiple correlations it is quite possible to get P-values of less than 0.05 by chance (1 out of 20). Using the Bonferroni correction only P-values of less than 0.0033 in table 9.8 and 0.0014 in table 9.9 should be regarded as significant at the 0.05 level. The results given above should therefore treated with caution.

9.4 DISCUSSION

We have measured indices of bone formation (osteocalcin and alkaline phosphatase) and resorption (urinary calcium:creatinine and hydroxyproline:creatinine ratios) in our group of 67 female athletes.

Indices of Bone resorption were lowest in those taking the oral contraceptive and highest in the amenorrhoeic athletes. Although this was seen with both measures of bone resorption it was only statistically significant with the urinary calcium:creatinine ratio. However this ratio is falling out of favour as a reliable marker of bone resorption. Furthermore there was no significant correlation between the 2 parameters of resorption. This may also reflect inaccuracies related to the collection of urinary hydroxyproline as a measure of bone resorption. For example, in

each subject the 24 hour gelatin-free diet may not have been strictly followed and the time of collection of the urine sample may have varied. The interpretation of these results should therefore be guarded.

The indices of Bone formation were also lowest in those taking the oral contraceptive. Although the highest mean alkaline phosphatase was found in the amenorrhoeic athletes, the highest mean osteocalcin was seen in the eumenorrhoeics. Osteocalcin is released solely from the osteoblast and is therefore a more specific marker of bone formation (Brown et al, 1984) than alkaline phosphatase (which is also released in large quantities from the liver). Despite this there was still a significant positive correlation between the 2 parameters of bone formation which has been seen in some, but not all, studies (chapter 2.4.2).

Serum osteocalcin levels vary with exercise acutely and chronically. Nishiyama et al (1988) and Bell et al (1988) showed that basal serum osteocalcin levels are elevated in young athletic male adults compared to sedentary controls whereas the alkaline phosphatase is unaltered. Nishiyama et al (1988) also showed that osteocalcin increases acutely (within an hour) in response to aerobic exercise while alkaline phosphatase remains unchanged. Their study suggests that serum osteocalcin and alkaline phosphatase represent different aspects of osteoblastic function. Some of the athletes in our study would have performed

intense exercise within the previous 12 hours. This may have increased the serum osteocalcin and so obscured any change related to oestrogen status.

In contrast to the above studies, Dalsky et al (1988) found that osteocalcin decreased with aerobic exercise over a 9 month period in a group of postmenopausal women. The disparity in the response of osteocalcin to exercise may be accounted for by the age and sex differences of the cohorts studied.

Thus Bone turnover appeared lowest amongst those taking the oral contraceptive. In normal young adults, formation and resorption are closely coupled and bone mass is maintained (chapter 2.4.3). The amenorrhoeic athletes had the highest levels of bone resorption but possibly not formation, which might suggest uncoupling and negative balance for bone mass. However we were unable to demonstrate any significant relationship between the measures of formation and of resorption even amongst the "normal oestrogen" status athletes and therefore these results need to be interpreted with caution. Marcus et al (1985) also found that serum alkaline phosphatase and urinary calcium excretion was higher in a group of amenorrhoeic athletes than in their eumenorrhoeic counterparts.

We measured the active (ie carboxylated) fraction of osteocalcin as well as the total amount in the serum to see if differences in oestrogen status would affect the carboxylation of the glutamic acid residues (chapter 2.4.2). We were unable to demonstrate any

differences between the 3 menstrual groups suggesting that oestrogen has little effect on the carboxylation process. The ratio of active:total osteocalcin was about 80% which is similar to that reported by Menon et al (1987).

Parathyroid hormone and 25-hydroxyvitamin D did not vary between the 3 menstrual groups. We were also unable to show any correlation between PTH, 25-hydroxyvitamin D and the parameters of bone turnover. Marcus et al (1985) were also unable to show differences in PTH and 25-hydroxyvitamin D between low and normal oestrogen status athletes, but they did show increased levels of 1,25 dihydroxyvitamin D in amenorrhoeic athletes. In contrast, Cook et al (1987) found that c-terminal midmolecule PTH was lower in a group of oligomenorrhoeic athletes compared to their eumenorrhoeic counterparts. The discrepancy may be due to differences in assay technique.

We also investigated the relationship between the biochemical and exercise parameters. We were unable to show any relationship between $\dot{V}O_2max$ and measures of bone formation. Ismail et al (1989) found a positive correlation between $\dot{V}O_2max$ and osteocalcin in a group of elderly subjects where, presumably, the range for $\dot{V}O_2max$ was much lower. We did show weak, positive correlations between $\dot{V}O_2max$ and the urinary calcium:creatinine ratio, a measure of bone resorption and between isokinetic strength for back extension and osteocalcin, a measure of bone

formation. However as we were looking at many linear correlations, these "significant" results may have occured by chance and may represent false positives.

9.5 CONCLUSIONS

1. Athletes on the oral contraceptive have reduced levels of alkaline phosphatase, osteocalcin and urinary calcium excretion suggesting a lower rate of bone turnover.

2. Amenorrhoeic athletes have increased levels of urinary calcium excretion and may have uncoupling of bone formation and resorption leading to negative balance of bone mass.

3. The ratio of active (carboxylated) osteocalcin to total osteocalcin is unaffected by changes in oestrogen status.

4. Aerobic fitness ($\dot{V}O_2max$) and isokinetic strength may be positively correlated to some parameters of bone turnover.

TABLE 9.1
SERUM BIOCHEMISTRY IN THE 25 ATHLETES WITH AMENORRHOEA.
(Row = rowers; Ru = runners; D = dancers)

REG. NO.	SPORT	CALCIUM (mmol/l)	ALBUMIN (g/l)	PHOSPHATE (mmol/l)	PTH (pmol/l)	25-OH Vit. D (nmol/l)
006	Row	2.32	48	.94	4.8	103
007	Row	2.48	52	1.34	2.1	115
008	Row	2.38	50	1.18	3.5	66
014	Row	2.46	50	1.17	3.1	82
019	Row	2.39	50	1.47	1.7	121
023	D	2.25	44	1.19	3.1	54
024	Row	2.49	54	1.18	2.9	131
026	D	2.27	45	1.32	3.5	84
027	Row	2.31	49	1.17	3.1	115
028	Row	2.29	46	1.07	2.1	69
031	D	2.41	48	.99	3.2	72
035	Row	2.47	50	1.13	.9	96
042	D	2.44	49	1.22	1.8	135
043	Ru	2.45	49	1.12	2.1	174
046	Ru	2.34	45	1.17	3.6	124
052	Ru	2.23	48	1.29	3.4	123
060	Ru	2.35	34	1.07	2.5	117
061	Row	2.10	39	1.07	6.9	95
062	Ru	2.28	44	1.33	4.4	88
063	Ru	2.37	41	1.16	2.7	174
064	Ru	2.30	38	1.22	3.4	192
065	Ru	2.32	44	.93	4.3	113
066	Ru	2.25	39	1.17	2.1	80
068	Ru	2.26	43	1.28	3.1	76
069	Ru	2.34	41	1.14	2.1	105

TABLE 9.3
SERUM BIOCHEMISTRY IN THE 15 ATHLETES ON THE ORAL CONTRACEPTIVE
(Row = rowers; Ru = runners; D = dancers)

REG. NO.	SPORT	CALCIUM (mmol/l)	ALBUMIN (g/l)	PHOSPHATE (mmol/l)	PTH (pmol/l)	25-OH Vit. D (nmol/l)
003	Row	2.27	45	1.07	2.1	105
005	Row	2.34	47	1.15	2.9	104
010	Row	2.33	47	1.18	2.9	113
012	Row	2.39	49	1.11	2.3	84
015	Row	2.31	46	1.01	3.1	108
022	Row	2.34	46	1.11	1.5	159
030	Row	2.22	45	1.11	2.7	60
034	Row	2.38	41	1.11	2.8	150
045	Row	2.35	44	1.16	3.1	135
047	Ru	2.25	44	.59	1.4	110
057	D	2.21	35	.93	3.9	110
004	Row	2.44	47	1.24	4.3	41
049	Ru	2.31	44	1.04	2.6	95
050	Ru	2.33	44	1.47	3.9	116
044	Row	2.46	49	1.11	2.2	140

TABLE 9.2
SERUM BIOCHEMISTRY IN THE 27 ATHLETES WITH EUMENORRHOEA
(Row = rowers; Ru = runners; D = dancers)

REG. NO.	SPORT	CALCIUM (mmol/l)	ALBUMIN (g/l)	PHOSPHATE (mmol/l)	PTH (pmol/l)	25-OH Vit. D (nmol/l)
001	Row	2.31	47	1.12	3.3	67
002	Row	2.48	49	1.22	3.1	194
009	Row	2.43	53	1.21	2.7	149
011	Row	2.29	48	1.27	5.3	73
016	Row	2.34	45	1.05	7.6	71
017	Row	2.45	50	1.15	1.5	66
018	Row	2.42	49	1.08	2.2	90
020	Row	2.34	58	1.13	2.9	132
021	Row	2.42	46	1.38	2.3	96
025	Row	2.32	44	.78	2.7	92
032	Row	2.36	47	1.33	2.9	70
033	Row	2.32	46	1.41	3.1	95
036	Row	2.41	48	1.08	2.4	109
037	D	2.49	49	1.19	2.6	112
038	D	2.35	47	1.18	6.1	58
039	Row	2.41	47	1.24	2.1	202
040	D	2.36	50	1.24	2.7	54
048	Ru	2.43	46	1.22	1.7	101
051	Ru	2.23	45	.93	1.8	150
053	Ru	2.58	44	1.04	2.2	100
054	Ru	2.38	42	1.08	2.2	114
055	D	2.32	46	1.25	2.5	93
056	D	2.27	41	1.53	1.5	95
058	Row	2.31	42	.86	2.6	51
059	Ru	2.30	40	.79	3.2	150
067	Ru	2.34	40	.88	4.1	77
070	Ru	2.22	41	1.08	2.9	68

TABLE 9.4
BONE TURNOVER PARAMETERS IN THE 25 AMENORRHOEIC ATHLETES

REG. NO.	URINARY Ca:Cr	URINARY Hproline :Cr	Alkaline Phos. (IU/L)	Osteocal. -total (ng/ml)	Osteocal. -active (ng/ml)
006	.43	.011	74	2.85	2.42
007	.37	.014	55	2.86	2.35
008	.25	.011	64	1.78	1.39
014	.94	.044	66	3.80	3.20
019	.83	.024	79	2.95	2.43
023	.48	.018	95	4.20	3.78
024	.41	.011	86	4.90	3.55
026	.33	.015	76	4.10	3.66
027	.52	.166	70	3.40	2.95
028	.25	.011	48	2.09	1.74
031	.27	.011	94	3.40	2.99
035	.57	.009	60	4.25	3.30
042	.31	.003	64	2.34	1.81
043	.26	.011	113	3.16	2.61
046	.57	.023	79	3.57	2.83
052	.43	.025	64	2.10	1.71
060	.46	.017	60	2.93	2.47
061	.42	.023	79	3.02	2.39
062	.20	.017	99	3.22	2.78
063	1.53	.017	85	2.29	1.84
064	1.04	.024	83	2.13	1.65
065	.95	.009	83	3.49	3.15
066	.18	.012	86	4.37	3.44
068	.83	.010	44	2.56	2.11
069	.41	.012	48	3.27	2.92

TABLE 9.6
BONE TURNOVER PARAMETERS IN THE 15 ATHLETES ON THE ORAL CONTRACEPTIVE.

REG. NO.	URINARY Ca:Cr	URINARY Hproline :Cr	Alkaline Phos. (IU/L)	Osteocal. -total (ng/ml)	Osteocal. -active (ng/ml)
003	.31	.017	42	1.12	.75
005	.16	.016	55	2.13	1.54
010	.51	.016	88	3.24	2.65
012	.35	.009	60	1.17	.78
015	.21	.011	74	2.95	2.61
022	.45	.008	51	1.75	1.25
030	1.09	.009	56	2.20	1.69
034	.19	.015	40	3	2.58
045	.03	.025	67	2.75	1.83
047	.24	.011	38	1.70	1.35
057	.32	.018	50	3.45	2.79
004	.23	.025	72	2.68	2.24
049	.24	.015	53	2.65	1.93
050	.11	.018	64	3.12	2.50
044	.26	.017	100	6.90	6.03

TABLE 9.5
BONE TURNOVER PARAMETERS IN THE 27 ATHLETES WITH EUMENORRHOEA.

REG. NO.	URINARY Ca:Cr	URINARY Hproline :Cr	Alkaline Phos. (IU/L)	Osteocal.-total (ng/ml)	Osteocal.-active (ng/ml)
001	.81	.035	52	2.29	1.89
002	.48	.018	94	3.76	3.33
009	.31	.014	72	4.20	3.68
011	.29	.032	64	5.30	4.73
016	.21	.011	59	4.80	4.31
017	.61	.012	78	3.85	3.18
018	.16	.011	56	3.12	2.46
020	.07	*	53	2.70	2.27
021	.41	.009	50	4	3.44
025	.82	.009	55	2.25	1.80
032	.29	.022	78	3.80	3.03
033	.23	.009	53	2.79	2.40
036	.33	.004	77	3.66	3.03
037	.31	.026	95	2.23	1.84
038	.99	.016	70	5.21	3.84
039	.44	*	64	3.50	2.98
040	.29	.008	58	3.40	2.50
048	.45	.014	49	3.45	2.48
051	.17	.013	57	2.90	2.46
053	.29	.021	72	5.25	4.07
054	.84	.021	58	2.88	2.07
055	.36	.030	476	4.10	3.31
056	.30	.046	78	6.60	5.32
058	.20	.014	57	3.80	3.24
059	.42	.023	85	1.81	1.30
067	.38	.010	136	3.27	2.73
070	.17	.018	50	3.69	2.94

* indicates missing data

TABLE 9.7:
MEAN (95% CONFIDENCE INTERVAL) PARAMETERS OF BONE BIOCHEMISTRY

	AMEN	EUMEN	ORAL CONTRACEPTIVE TAKERS	ANOVA
Number	25	27	15	
Active Osteocalcin (ng/ml)	2.53 (2.19,2.92)	2.83 (2.47,3.25)	1.90 (1.58,2.29)	F = 6.03 P = 0.0040
Total Osteocalcin (ng/ml)	3.06 (2.69,3.48)	3.49 (3.08,3.94)	2.47 (2.09,2.92)	F = 5.54 P = 0.0060
Alkaline Phosphatase (IU/l)	72.2 (65.4,79.8)	65.9 (59.9,72.5)	58.5 (51.4,66.5)	F = 3.38 P = 0.040
Urinary Ca: Cr Ratio	0.455 (0.355,0.583)	0.337 (0.266,0.425)	0.244 (0.177,0.335)	F = 4.87 P = 0.011
Urinary Hydroxypro-line: Cr Ratio	0.0156 (0.0124,0.0196)	0.0153 (0.0122,0.0192)	0.0145 (0.0108,0.0196)	F = 0.07 P = 0.93
25 Hydroxy-Vitamin D (nmol/l)	103 (90,118)	94 (82,107)	103 (87,123)	F = 0.66 P = 0.52
PTH (pmol/l)	2.84 (2.43,3.31)	2.81 (2.43,3.25)	2.66 (2.18,3.24)	F = 0.15 P = 0.86

In all cases, the ANOVA was performed on logs of data to ensure the residuals had equal variances in the three menstrual states and Normal distribution.

TABLE 9.8: CORRELATIONS (P-VALUE) BETWEEN BIOCHEMICAL PARAMETERS

	Osteocalcin (Total)	Urinary Ca:Cr	Urinary Hyd. Cr	PTH	Vitamin D
Alkaline Phosphatase	0.313 **(0.010)**	0.081 (0.51)	0.049 (0.70)	0.179 (0.15)	0.152 (0.22)
Osteocalcin (Total)	-	-0.099 (0.42)	0.114 (0.36)	0.132 (0.28)	-0.126 (0.31)
Urinary Ca:Cr	-	-	0.089 (0.48)	0.034 (0.79)	0.107 (0.38)
Urinary Hyd:Cr	-	-	-	0.053 (0.67)	0.038 (0.76)
PTH	-	-	-	-	-0.265 **(0.029)**

TABLE 9.9: CORRELATIONS (P-VALUE) BETWEEN THE BIOCHEMICAL AND EXERCISE PARAMETERS

	$\dot{V}O_2$ Max	Body Fat	Isokinetic Back Strength	
			Flexion	Extension
Alkaline Phosphatase	0.072 (0.56)	-0.145 (0.24)	0.061 (0.63)	-0.167 (0.18)
Osteocalcin (Total)	-0.193 (0.12)	0.044 (0.72)	0.144 (0.25)	0.275 **(0.026)**
Urinary Ca:Cr	0.284 **(0.020)**	-0.165 (0.18)	-0.126 (0.31)	-0.113 (0.37)
Urinary Hyd:Cr	0.056 (0.66)	-0.113 (0.37)	0.066 (0.61)	-0.039 (0.76)
PTH	-0.065 (0.60)	0.180 (0.14)	0.048 (0.70)	0.048 (0.70)
Vitamin D	0.189 (0.13)	-0.118 (0.34)	-0.121 (0.33)	-0.138 (0.27)

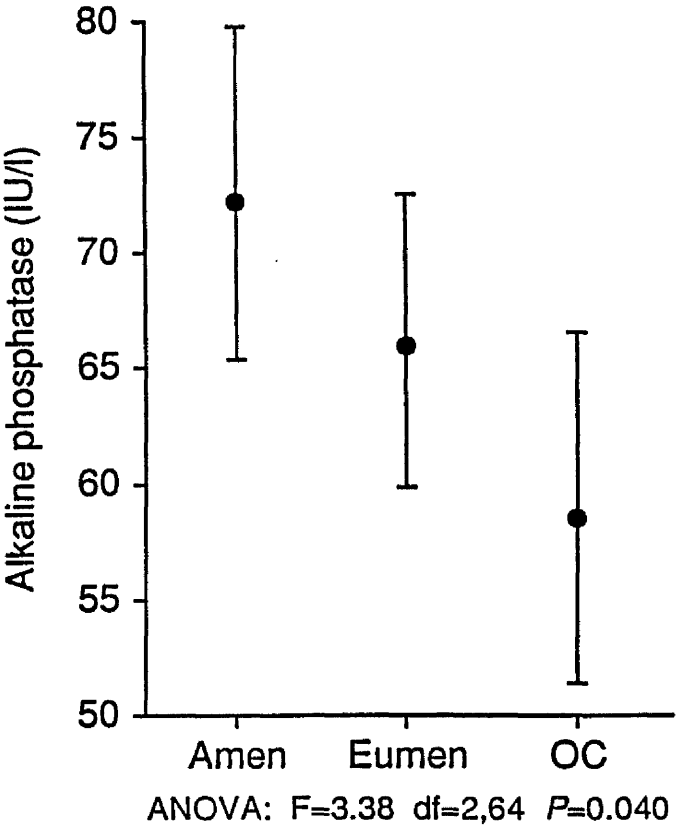

Figure 9.1:

Mean (95% Confidence Interval) Alkaline Phosphatase (IU/l) in the Amenorrhoeic, Eumenorrhoeic and Oral Contraceptive-taking Athletes.

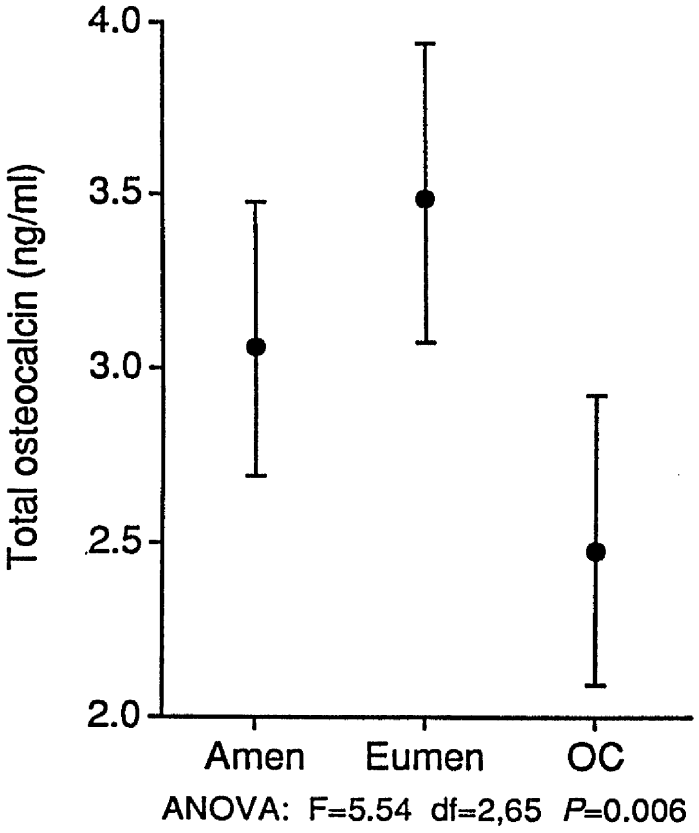

Figure 9.2:

Mean (95% Confidence Interval) Total Osteocalcin (ng/ml) in the Amenorrhoeic, Eumenorrhoeic and Oral Contraceptive-taking Athletes.

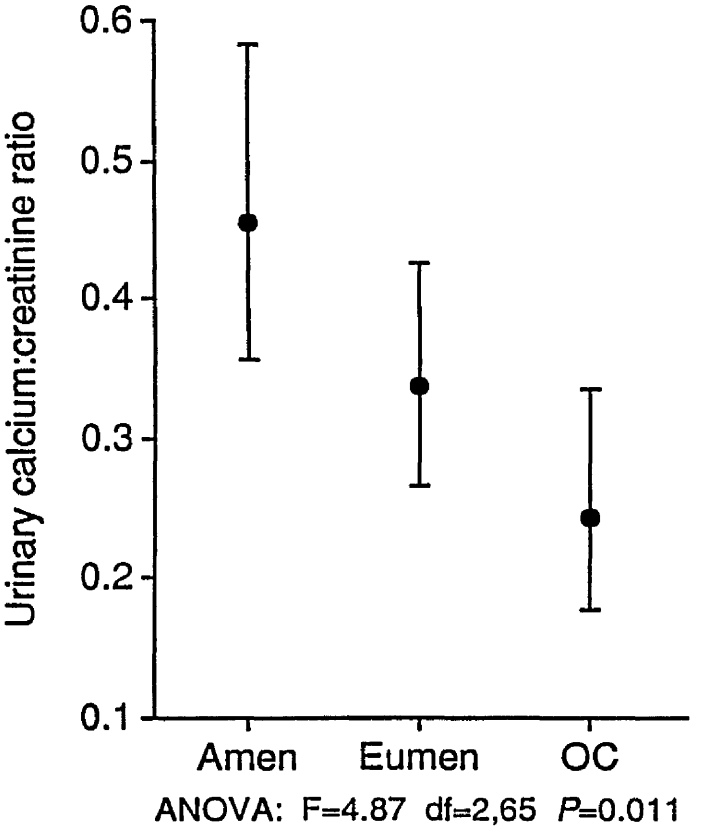

Figure 9.3:

Mean (95% Confidence Interval) Urinary Calcium:Creatinine Ratio in the Amenorrhoeic, Eumenorrhoeic and Oral Contraceptive-taking Athletes.

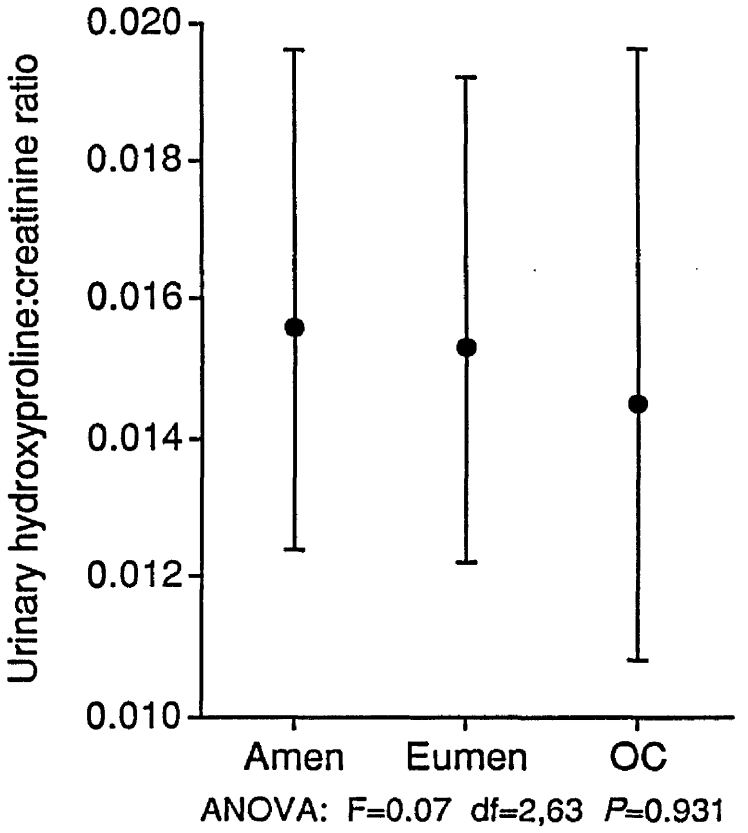

Figure 9.4:

Mean (95% Confidence Interval) Urinary Hydroxyproline: Creatinine Ratio in the Amenorrhoeic, Eumenorrhoeic and Oral Contraceptive-taking Athletes.

CHAPTER 10: RESULTS AT FOLLOW UP

10.1 THE COHORT

Sixty-two of the original 67 athletes agreed to return for further testing, one year later. The following did not return:-

038	Dancer	because	left the country
039	Rower	"	lack of interest
045	Rower	"	pregnant
067	Runner	"	lack of interest
069	Runner	"	pregnant.

The remaining 62 athletes kept a menstrual diary throughout the year. Tables 10.1.1, 10.1.2 and 10.1.3 give the menstrual status of each athlete at the start of the study, the number of periods each had during the ensuing year and the menstrual status at the end of the year, when they returned for follow up. The definitions of menstrual status are given in chapter 3.3. One subject (036) completed a full term pregnancy in between her first and second attendances. Table 10.1.4 shows distribution of the menstrual states at the first and second attendance. The number in each menstrual status category at the second attendance was as follows:-

Amenorrhoeics	15
Oligomenorrhoeics	8
Eumenorrhoeics	20
Oral Contraceptive takers	18
Completed Pregnancy	1.

This cohort consisted of athletes from the following sports:-

Dancers	9
Rowers	33
Runners	19.

One of the rowers (007) had retired from rowing and had taken up running during the course of the year.

Training intensity was also defined at the return visit. Each athlete was asked to state whether their training intensity had increased, not changed or decreased compared to the time of their first attendance. The results from each of the rowers, runners and dancers are given in tables 10.1.1, 10.1.2 and 10.1.3 respectively. Amongst the runners, training intensity was also defined by the average number of miles run per week over the previous 6 months (table 10.1.2).

One rower (008) and two runners (060 and 062) had to decrease their training substantially due to injury.

We repeated the original bone mineral density measurements (femoral midshaft and lumbar spine) on each of the 62 subjects (chapter 10.2). We also measured the bone mineral density of the left proximal femur in each subject (chapter 10.3).

TABLE 10.1.1
MENSTRUAL STATUS AT FIRST AND SECOND ATTENDANCE AMONGST THE ROWERS
(A= amenorrhoea; O= oligomenorrhoea; E= eumenorrhoea; C= oral contraceptive; P= pregnant)
(D= decreased; N= no change; I= increased)

REG. NO.	MEN.STAT (1st attend.)	MEN.STAT (2nd attend.)	No of Periods during yr	Training Intensity
007*	A	A	2	N
008	A	A	1	D
024	A	A	0	D
028	A	O	6	N
061	A	O	6	I
006	A	E	9	D
019	A	E	10	D
027	A	E	10	N
015	A	C	12	D
035	A	C	10	N
036	E	P	0	D
002	E	O	7	N
011	E	O	6	D
025	E	O	4	I
009	E	E	10	D
017	E	E	10	N
020	E	E	12	D
021	E	E	11	D
032	E	E	12	I
033	E	E	11	I
058	E	E	11	I
001	E	C	12	D
016	E	C	11	I
018	E	C	12	D
014	C	O	7	N
004	C	E	12	N
003	C	C	12	D
005	C	C	12	I
010	C	C	8	N
012	C	C	11	N
022	C	C	12	N
030	C	C	12	N
034	C	C	12	D
044	C	C	12	N

* Ex-Rower, now running

TABLE 10.1.2
MENSTRUAL STATUS AT FIRST AND SECOND ATTENDANCE AMONGST THE RUNNERS
(A= amenorrhoea; O= oligomenorrhoea; E= eumenorrhoea; C= oral contraceptive; P= pregnant)
(D= decreased; N= no change; I= increased)

REG. NO.	MEN.STAT (1st attend.)	MEN.STAT (2nd attend.)	No of Periods during yr	Training Intensity	Miles Run per Wk
043	A	A	1	I	70
046	A	A	0	D	30
052	A	A	0	I	50
060	A	A	0	D	0
063	A	A	0	N	50
064	A	A	0	N	45
066	A	A	0	I	50
068	A	A	1	N	70
062	A	E	10	D	0
065	A	E	8	D	50
048	E	O	9	I	80
054	E	O	7	N	30
051	E	E	12	D	5
059	E	E	11	N	35
070	E	E	12	N	35
053	E	C	8	I	45
047	C	E	12	I	37
049	C	C	0	I	55
050	C	C	0	D	25

TABLE 10.1.3
MENSTRUAL STATUS AT FIRST AND SECOND ATTENDANCE AMONGST THE DANCERS
(A= amenorrhoea; O= oligomenorrhoea; E= eumenorrhoea; C= oral contraceptive; P= pregnant)
(D= decreased; N= no change; I= increased)

REG. NO.	MEN.STAT (1st attend.)	MEN.STAT (2nd attend.)	No of Periods during yr	Training Intensity
023	A	A	0	N
026	A	A	3	N
031	A	A	1	I
042	A	A	0	N
040	E	E	13	I
055	E	E	11	N
056	E	E	12	N
037	E	C	12	N
057	C	C	12	N

TABLE 10.1.4 : Menstrual Status in the 62 Athletes at their First and Second Attendance

		FIRST YEAR			
		Amen	Eumen	Oral Contraceptive	**TOTAL**
SECOND YEAR	Amen	15	0	0	15
	Oligomen.	3	5	0	8
	Eumen	5	13	2	20
	Oral Contraceptive	1	5	12	18
	Pregnant	0	1	0	1
TOTAL		24	24	14	62

10.2 LONGITUDINAL CHANGES IN BONE DENSITY

10.2.1 INTRODUCTION

The bone density changes seen in amenorrhoeic athletes are mainly based on cross-sectional data. Two longitudinal studies have been performed (chapter 2.3.5) but these involved only small groups, making interpretation of the data difficult.

We have remeasured the bone mineral density in the femoral midshaft and lumbar spine in 62 female athletes, one year after their original measurement.

10.2.2 METHOD

Out of the original 67 athletes, 5 dropped out during the year and 62 returned for follow up (chapter 10.1). Each athlete kept a record of their menstrual pattern throughout the year which enabled us to define their menstrual status when they returned. Table 10.1.4 shows the distribution of the menstrual states at the first and second attendance. From this we were able to define 4 categories regarding the first and second attendance (the number in each group is also given):-

1) AA Athletes who remained amenorrhoeic 15;

2) NN Athletes whose oestrogen status remained "normal", ie eumenorrhoeics and oral contraceptive takers 32;

3) AON Athletes who were initially amenorrhoeic but became oligomenorrhoeic or "normal" oestrogen status, ie oestrogen status improved 9;

4) NO Athletes whose oestrogen status was initially "normal" but who became oligomenorrhoeic, ie oestrogen status worsened 5.

Training intensity in comparison to the first attendance was assessed for each athlete (chapter 10.1). For each runner the average number of miles run per week over the previous 6 months was documented.

The daily calcium intake questionnaire (chapter 3.7) was repeated in each athlete and the change in intake over the year was also recorded.

The measurements of trabecular bone density of the lumbar spine and the bone mineral content of the right femoral midshaft (chapter 3.4) were repeated in each athlete.

Statistical Methods

Analysis of variance was used to determine whether the change in bone density at each of the 2 sites differed between the 4 menstrual categories. Analysis of variance was also used to investigate the relationship between the changes in spinal trabecular bone density and training intensity amongst the rowers. The relationship between the change in femoral shaft bone mineral content and the miles run per week amongst the runners was investigated using linear correlations.

We investigated the relationship between the change in bone density and the following 5 variables:-

a) change in menstrual status over the year,

b) change in training intensity over the year,

c) change in calcium intake over the year,

d) original sporting group and

e) original $\dot{V}O_2max$ (chapter 7).

Linear models (McCullagh and Nelder, 1983; GLIM, 1986) were used to assess which of these 5 variables, or combination of them, could best explain the variation in bone mineral density at each of the 2 sites (see "Statistical Methods", chapter 7.2).

10.2.3 RESULTS

Tables 10.2.1, 10.2.3, 10.2.5 and 10.2.7 give the spinal bone density measurements for the first and second attendance for each athlete in groups AA, NN, AON and NO respectively. Tables 10.2.2, 10.2.4, 10.2.6 and 10.2.8 give the femoral shaft bone density measurements for the first and second year for each athlete in each of these groups. Table 10.2.9 gives the mean (95% confidence interval) change in spinal trabecular bone density and femoral shaft bone mineral content in each of the 4 menstrual categories. There was no significant difference between the 4 groups at either skeletal site (P= 0.17 for the spine; P= 0.52 for the femoral shaft) - figures 10.2.1 and 10.2.2.

Spinal trabecular bone density changed by more than 30% in some of the athletes (increases of 31% in 026, 32% in 068 and 37% in 016). Cortical bone density in the femoral midshaft however changed by no more than 9% (the 4 athletes with the greatest increases were 9% in 014 and 048, 8% in 056 and 7% in 007). Subject number 016 was a rower who had increased her training substantially over the year after an injury and had an associated large increase in spinal bone density. Subject number 007 was

initially a rower but became a runner during the year running, on average, 40 miles/week. She had a large associated increase in cortical bone density in the femoral midshaft. However, despite these features, in the runners there was no overall relationship between the changes in femoral shaft bone density and training intensity (P= 0.75) or miles run/week (P= 0.76) or in the rowers between the changes in spinal bone density and training intensity (P= 0.51).

Tables 10.2.10, 10.2.11, 10.2.12 and 10.2.13 give the daily calcium intake for the first and second attendance and the original sporting category of each athlete in groups AA, NN, AON and NO respectively. Tables 7.1, 7.2 and 7.3 give the original $\dot{V}O_2$max measurements in each athlete.

Table 10.2.14 gives the results of model building for the changes in lumbar spine and femoral shaft bone densities. None of the 5 variables investigated was significantly related to the change in bone density at either site.

10.2.4 DISCUSSION

We have followed up 61 female athletes for 1 year and monitored the change in bone density at 2 different sites during that time period. Although individual changes were large in some cases, the overall change in the 15 athletes who remained amenorrhoeic and the 32 whose oestrogen status remained "normal" was minimal.

There was a fall in trabecular bone density amongst the 5 athletes whose oestrogen status decreased. However the difference between the changes in this menstrual category and the other 3 was not statistically significant. This may be due to substantial variability in each of the menstrual groups suggested by the wide confidence intervals. We were also unable to demonstrate any significant relationship between 4 other parameters and the change in spinal trabecular bone density which again may be due to the large variability.

In contrast to the wide fluctuations in trabecular bone density changes, there were only small changes in cortical bone density of the femoral midshaft over the year. The changes seen were similar in each of the 4 menstrual categories. There was also no significant relationship between the change in cortical bone density and any of the 4 other parameters. The relationship between training intensity and spinal bone density in the rowers and femoral shaft bone density in the runners was also not significant. However follow up for only 1 year may not be sufficient time to demonstrate important changes.

The lack of relationship between any of the parameters and the bone density at either site suggests that in addition to the large variability in spinal trabecular bone density changes, the time length of follow up may not have been sufficiently long to reveal specific trends.

Other longitudinal studies on athletic amenorrhoea have been performed. Drinkwater et al (1986) restudied 9 of the 14 amenorrhoeic and 7 of the 14 eumenorrhoeic athletes from their original study (Drinkwater et al, 1984). The lumbar spine BMD was measured 16 months after the original measurements. Seven of the 9 amenorrhoeic athletes had regained menses and in this group the BMD had increased by 6.3%. In the 2 who remained amenorrhoeic it decreased by 3.4%. There was no change in BMD in the women who remained eumenorrhoeic. Lindberg et al (1987) showed that in a 15 month period, spinal BMD in 4 athletes who regained their menses increased by an average of 6.6%, whereas in 3 who remained amenorrhoeic it decreased by an average of 1.3%. These studies had a longer follow up time than our own which may be an important factor in demonstrating trends but the cohort in both was small and hence the results may be less reliable. Dual photon absorptiometry was used to measure spinal bone density in both investigations and so they may not be directly comparable to our study.

10.2.5 CONCLUSIONS

1. There are wide fluctuations in spinal trabecular bone density over the course of one year.

2. Fluctuations of cortical bone density in the femoral midshaft were much less dramatic.

3. Although there was a fall in trabecular bone density amongst the athletes whose oestrogen status decreased, this was not significantly different from the athletes whose menstrual status remained unchanged.

4. Cortical bone density in the femoral midshaft was unaffected by changes in oestrogen status over the year.

5. Changes in training intensity and in calcium intake were unrelated to the changes in bone density at either site.

TABLE 10.2.1
CHANGES IN SPINAL TRABECULAR BONE DENSITY OVER ONE YEAR IN ATHLETES WHO REMAINED AMENORRHOEIC (AA).
(Da= dancer; Ro= rower; Ru= runner; D= decreased; N= no change; I= increased)

REG.NO.	SPORT	CHANGE IN INTENSITY	SPINAL TBD (mg/cm^3) 1st yr	2nd yr	2nd-1st
007	Ru -ex Ro	N	216	213	-3
008	Ro	D	154	148	-6
023	Da	N	168	156	-13
024	Ro	D	185	174	-11
026	Da	N	115	151	36
031	Da	I	121	147	26
042	Da	N	144	163	19
043	Ru	I	221	212	-10
046	Ru	D	148	132	-16
052	Ru	I	149	126	-23
060	Ru	D	221	197	-24
063	Ru	N	96	107	11
064	Ru	N	105	108	2
066	Ru	I	115	144	29
068	Ru	N	149	198	48

TABLE 10.2.2
CHANGE IN FEMORAL SHAFT BONE MIN. CONTENT OVER ONE YEAR IN ATHLETES WHO REMAINED AMENORRHOEIC (AA).
(Da= dancer; Ro= rower; Ru= runner; D= decreased; N= no change; I= increased)

REG.NO.	SPORT	CHANGE IN INTENSITY	FEM. SHAFT BMC (gm/cm^2) 1st yr	2nd yr	2nd-1st
007	Ru -ex Ro	N	1.49	1.59	.10
008	Ro	D	1.34	1.32	-.02
023	Da	N	1.34	1.37	.03
024	Ro	D	1.29	1.32	.03
026	Da	N	1.27	1.29	.02
031	Da	I	1.31	1.32	.01
042	Da	N	1.46	1.51	.05
043	Ru	I	1.62	1.63	.01
046	Ru	D	1.29	1.29	0
052	Ru	I	1.61	1.54	-.07
060	Ru	D	1.58	1.60	.02
063	Ru	N	1.44	1.46	.02
064	Ru	N	1.48	1.47	-.01
066	Ru	I	1.46	1.41	-.05
068	Ru	N	1.48	1.45	-.03

TABLE 10.2.3
CHANGE IN SPINAL TRABECULAR BONE DENSITY OVER ONE YEAR IN ATHLETES WHOSE OESTROGEN STATUS REMAINED NORMAL (NN).
(Da= dancer; Ro= rower; Ru= runner; D= decreased; N= no change; I= increased)

REG.NO.	SPORT	CHANGE IN INTENSITY	SPINAL TBD (mg/cm^3) 1st yr	2nd yr	2nd-1st
001	Ro	D	193	181	-12
003	Ro	D	256	208	-48
004	Ro	N	252	210	-42
005	Ro	I	252	219	-33
009	Ro	D	242	206	-36
010	Ro	N	234	267	33
012	Ro	N	221	226	5
015	Ro	D	239	212	-27
016	Ro	I	187	256	69
017	Ro	N	210	230	20
018	Ro	D	184	202	18
020	Ro	D	196	202	6
021	Ro	D	198	179	-19
022	Ro	N	185	139	-46
030	Ro	N	255	264	9
032	Ro	I	238	245	7
033	Ro	I	199	193	-6
034	Ro	D	212	248	36
037	Da	N	199	202	3
040	Da	I	214	221	7
044	Ro	N	209	192	-17
047	Ru	I	178	167	-11
049	Ru	I	224	176	-48
050	Ru	D	140	147	7
051	Ru	D	223	199	-24
053	Ru	I	247	240	-7
055	Da	N	206	207	1
056	Da	N	180	162	-18
057	Da	N	215	214	-1
058	Ro	I	224	218	-6
059	Ru	N	266	237	-29
070	Ru	I	197	211	14

TABLE 10.2.4
CHANGE IN FEMORAL SHAFT BONE MIN. CONTENT OVER ONE YEAR IN ATHLETES WHOSE OESTROGEN STATUS REMAINED NORMAL (NN).
(Da= dancer; Ro= rower; Ru= runner; D= decreased; N= no change; I= increased)

REG.NO.	SPORT	CHANGE IN INTENSITY	FEM. SHAFT BMC (gm/cm^2) 1st yr	2nd yr	2nd-1st
001	Ro	D	1.39	1.46	.07
003	Ro	D	1.42	1.46	.04
004	Ro	N	1.31	1.35	.04
005	Ro	I	1.50	1.55	.05
009	Ro	D	1.41	1.45	.04
010	Ro	N	1.44	1.43	-.01
012	Ro	N	1.46	1.46	0
015	Ro	D	1.45	1.47	.02
016	Ro	I	1.48	1.50	.02
017	Ro	N	1.41	1.37	-.04
018	Ro	D	1.55	1.49	-.06
020	Ro	D	1.59	1.61	.02
021	Ro	D	1.46	1.43	-.03
022	Ro	N	1.41	1.44	.03
030	Ro	N	1.42	1.41	-.01
032	Ro	I	1.43	1.40	-.03
033	Ro	I	1.50	1.42	-.08
034	Ro	D	1.49	1.57	.08
037	Da	N	1.42	1.39	-.03
040	Da	I	1.45	1.38	-.07
044	Ro	N	1.48	1.44	-.04
047	Ru	I	1.48	1.49	.01
049	Ru	I	1.62	1.59	-.03
050	Ru	D	1.39	1.38	-.01
051	Ru	D	1.68	1.73	.05
053	Ru	I	1.44	1.48	.04
055	Da	N	1.35	1.37	.02
056	Da	N	1.39	1.50	.11
057	Da	N	1.52	1.48	-.04
058	Ro	I	1.47	1.56	.09
059	Ru	N	1.53	1.55	.02
070	Ru	I	1.48	1.50	.02

TABLE 10.2.5
CHANGES IN SPINAL TRABECULAR BONE DENSITY OVER ONE YEAR
IN ATHLETES WHOSE OESTROGEN STATUS IMPROVED (AON).
(Da= dancer; Ro= rower; Ru= runner; D= decreased;
N= no change; I= increased; A= amenorrhoeic;
O= oligomenorrhoeic; N= normal oestrogen status)

REG.NO	SPORT	CHANGE IN INTENSITY	CHANGE IN OEST.STAT.	SPINAL TBD (mg/cm^3) 1st yr	2nd yr	2nd-1st
014	Ro	N	A-O	176	184	8
028	Ro	N	A-O	200	219	19
061	Ro	I	A-O	191	194	3
006	Ro	D	A-N	188	183	-5
019	Ro	D	A-N	191	195	4
027	Ro	N	A-N	160	171	11
035	Ro	N	A-N	197	238	41
062	Ru	D	A-N	198	156	-42
065	Ru	D	A-N	226	194	-32

TABLE 10.2.6
CHANGE IN FEMORAL SHAFT BONE MIN. CONTENT OVER ONE YEAR
IN ATHLETES WHOSE OESTROGEN STATUS IMPROVED (AON).
(Da= dancer; Ro= rower; Ru= runner; D= decreased;
N= no change; I= increased; A= amenorrhoeic;
O= oligomenorrhoeic; N= normal oestrogen status)

REG.NO	SPORT	CHANGE IN INTENSITY	CHANGE IN OEST.STAT.	FEM. SHAFT BMC (gm/cm^2) 1st yr	2nd yr	2nd-1st
014	Ro	N	A-O	1.20	1.31	.11
028	Ro	N	A-O	1.45	1.43	-.02
061	Ro	I	A-O	1.69	1.68	-.01
006	Ro	D	A-N	1.32	1.33	.01
019	Ro	D	A-N	1.41	1.45	.04
027	Ro	N	A-N	1.47	1.40	-.07
035	Ro	N	A-N	1.39	1.41	.02
062	Ru	D	A-N	1.63	1.64	.01
065	Ru	D	A-N	1.45	1.43	-.02

TABLE 10.2.7
CHANGES IN SPINAL TRABECULAR BONE DENSITY OVER ONE YEAR IN ATHLETES WHOSE OESTROGEN STATUS DECREASED (NO).
(Da= dancer; Ro= rower; Ru= runner; D= decreased; N= no change; I= increased)

REG.NO	SPORT	CHANGE IN INTENSITY	SPINAL TBD (mg/cm^3) 1st yr	2nd yr	2nd-1st
002	Ro	N	276	232	-44
011	Ro	D	181	167	-14
025	Ro	I	196	190	-6
048	Ru	I	178	164	-14
054	Ru	N	210	171	-39

TABLE 10.2.8
CHANGE IN FEMORAL SHAFT BONE MIN. CONTENT OVER ONE YEAR IN ATHLETES WHOSE OESTROGEN STATUS DECREASED (NO).
(Da= dancer; Ro= rower; Ru= runner; D= decreased; N= no change; I= increased)

REG.NO	SPORT	CHANGE IN INTENSITY	FEM. SHAFT BMC (gm/cm^2) 1st yr	2nd yr	2nd-1st
002	Ro	N	1.44	1.51	.07
011	Ro	D	1.23	1.27	.04
025	Ro	I	1.40	1.37	-.03
048	Ru	I	1.41	1.53	.12
054	Ru	N	1.55	1.55	0

Table 10.2.9: Mean (95% Confidence Interval) Change in Bone Mineral Density

Menstrual Group	Number of Subjects	Lumbar Spine (mg/cm^3)	Femoral Shaft (gm/cm^2)
AA	15	4.4 (-8.4,17.2)	0.0073 (-0.017,0.032)
AON	9	0.6 (-15.9,17.1)	0.0056 (-0.026,0.037)
NO	5	-23.1 (-45.3,-0.1)	0.0400 (-0.002,0.082)
NN	32	-6.1 (-14.9,2.7)	0.0075 (-0.009,0.024)
ANOVA	$F_{3,57}$	1.76	0.76
	P	0.17	0.52

TABLE 10.2.10

CHANGES IN DAILY CALCIUM INTAKE OVER ONE YEAR IN ATHLETES WHO REMAINED AMENORRHOEIC (AA).
(Da= dancer; Ro= rower; Ru= runner)

REG.NO.	SPORT	DAILY CALCIUM INTAKE (mg)		
		1st yr	2nd yr	2nd-1st
007	Ro	1376	521	-855
008	Ro	440	676	236
023	Da	975	1062	87
024	Ro	723	900	177
026	Da	279	527	248
031	Da	578	346	-232
042	Da	801	571	-230
043	Ru	405	671	266
046	Ru	863	506	-357
052	Ru	536	464	-72
060	Ru	551	682	131
063	Ru	580	584	4
064	Ru	551	529	-22
066	Ru	452	367	-85
068	Ru	1022	981	-41

TABLE 10.2.11

CHANGES IN DAILY CALCIUM INTAKE OVER ONE YEAR IN ATHLETES WHOSE OESTROGEN STATUS REMAINED NORMAL (NN).
(Da= dancer; Ro= rower; Ru= runner)

REG.NO.	SPORT	DAILY CALCIUM INTAKE (mg)		
		1st yr	2nd yr	2nd-1st
001	Ro	590	444	-146
003	Ro	601	713	112
004	Ro	1586	1541	-45
005	Ro	706	1267	561
009	Ro	989	641	-348
010	Ro	1110	772	-338
012	Ro	481	701	220
015	Ro	612	417	-195
016	Ro	893	1125	232
017	Ro	710	1266	556
018	Ro	833	552	-281
020	Ro	554	980	426
021	Ro	1002	257	-745
022	Ro	384	487	103
030	Ro	813	1245	432
032	Ro	872	935	63
033	Ro	500	269	-231
034	Ro	644	604	-40
037	Da	699	573	-126
040	Da	497	644	147
044	Ro	983	925	-58
047	Ru	454	801	347
049	Ru	640	900	260
050	Ru	313	196	-117
051	Ru	130	298	168
053	Ru	621	866	245
055	Da	490	509	19
056	Da	688	582	-106
057	Da	732	597	-135
058	Ro	502	769	267
059	Ru	506	540	34
070	Ru	708	1084	376

TABLE 10.2.12
CHANGES IN DAILY CALCIUM INTAKE OVER ONE YEAR IN ATHLETES WHOSE OESTROGEN STATUS IMPROVED (AON).
(Da= dancer; Ro= rower; Ru= runner)

REG.NO.	SPORT	DAILY CALCIUM INTAKE (mg)		
		1st yr	2nd yr	2nd-1st
006	Ro	1097	1043	-54
014	Ro	932	1065	133
019	Ro	248	238	-10
027	Ro	590	897	307
028	Ro	855	481	-374
035	Ro	1540	1671	131
061	Ro	1002	451	-551
062	Ru	1240	1708	468
065	Ru	808	710	-98

TABLE 10.2.13
CHANGES IN DAILY CALCIUM INTAKE OVER ONE YEAR IN ATHLETES WHOSE OESTROGEN STATUS DECREASED (NO).
(Da= dancer; Ro= rower; Ru= runner)

REG.NO.	SPORT	DAILY CALCIUM INTAKE (mg)		
		1st yr	2nd yr	2nd-1st
002	Ro	1724	2655	931
011	Ro	225	430	205
025	Ro	375	380	5
048	Ru	892	713	-179
054	Ru	725	390	-335

TABLE 10.2.14 : Model Fitting : CHANGES IN BONE DENSITY

Variables	Lumbar Spine (P value)	Femoral Shaft (P value)
Change in Men. Stat.	0.17	0.52
Change in Exercise Intensity	0.15	0.73
Change in Calcium Intake	0.53	0.87
Original Sport	0.21	0.84
$\dot{V}O_2$ **max**	0.44	0.17
$\dot{V}O_2$ **max (Quadratic)**	0.48	0.14

Values in each column represent the probability from the significance test of the relationship between each single variable and the change in bone density at each of the two sites. No P-value is <0.05.

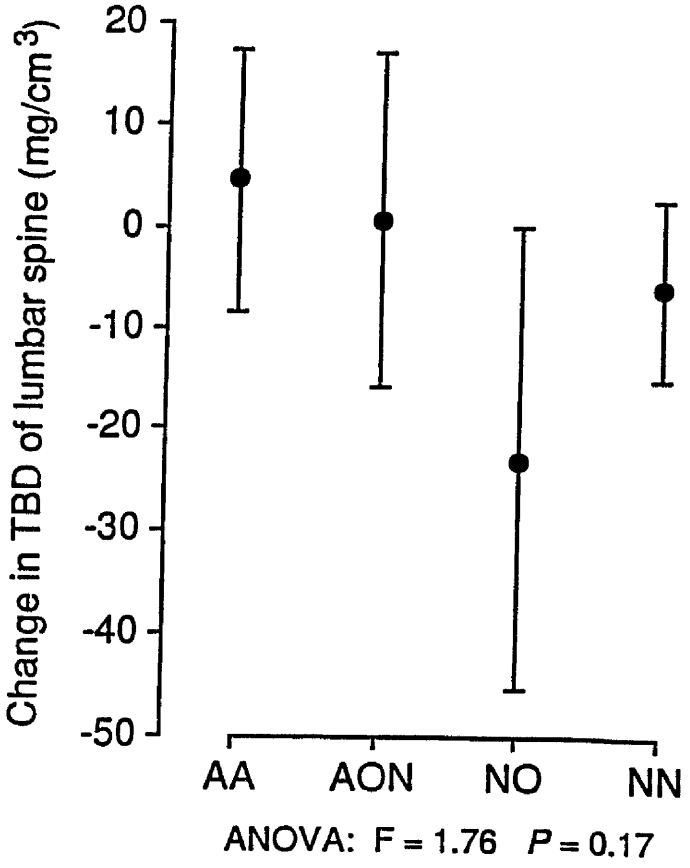

FIGURE 10.2.1

Changes in Spinal Trabecular Bone Density in the Athletes who remained amenorrhoeic (AA), the athletes whose oestrogen status improved (AON), the athletes whose oestrogen status decreased (NO) and the athletes whose oestrogen status remained normal (NN).

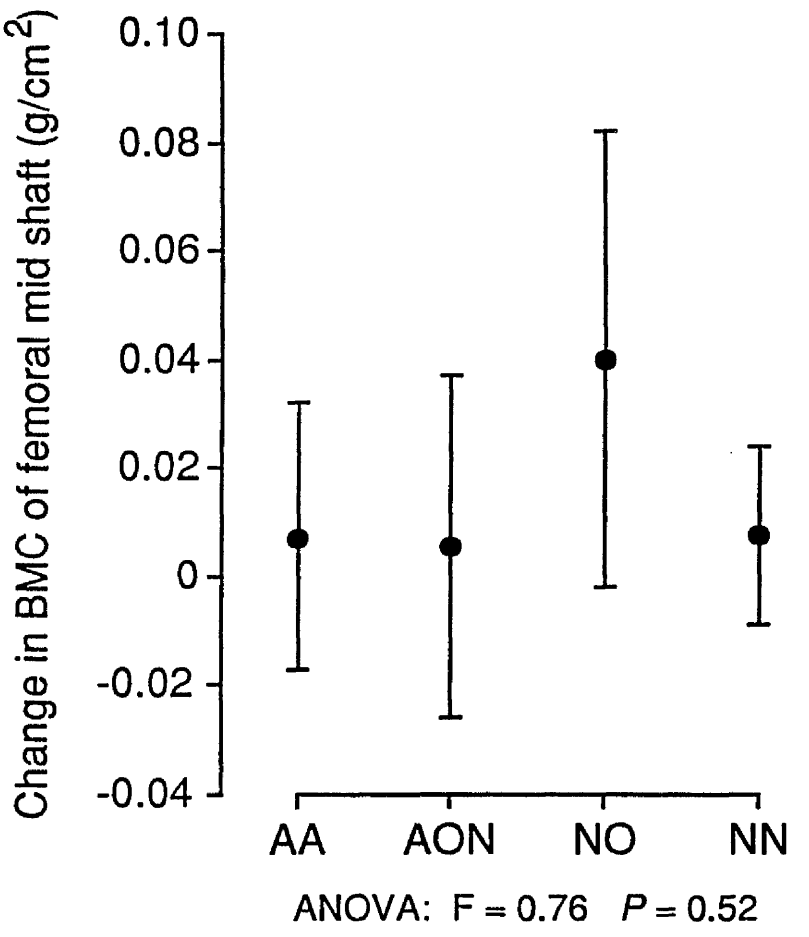

FIGURE 10.2.2:

Changes in the Bone Mineral Content of the Femoral Shaft in the Athletes who remained amenorrhoeic (AA), the athletes whose oestrogen status improved (AON), the athletes whose oestrogen status decreased (NO) and the athletes whose oestrogen status remain normal (NN).

10.3 BONE DENSITY OF THE LEFT PROXIMAL FEMUR

10.3.1 INTRODUCTION

The proximal femur consists of both cortical and trabecular bone, although cortical bone predominates. We have measured the bone mineral content at 3 sites within the hip and investigated the effect of menstrual status and of sporting activity at these sites.

10.3.2 METHOD

The study group comprised 61 female athletes, ie all the athletes who returned for the second attendance excluding one (036) who had completed a full term pregnancy (chapter 10.1). This group consisted of the following (the definitions of menstrual status are given in chapter 3.3):-

	Dancers	Rowers	Runners	TOTAL
Amenorrhoeics	4	3	8	15
Oligomenorrhoeics	-	6	2	8
Eumenorrhoeics	3	11	6	20
Oral Contracept. takers	2	13	3	18
TOTAL	9	33	19	61.

We measured the bone mineral content in the left hip by Dual Energy X-ray Absorptiometry as described in chapter 3.4.2. Measurements from the femoral neck, Ward's triangle and trochanteric regions were recorded (figure 3.4.1).

The measurements of height, weight and body fat content were repeated as described in chapter 3.5.

Statistical Methods

Analysis of variance was used to determine whether the bone density at each of the 3 sites varied between the menstrual and sporting groups.

The relationship between bone density of the hip and age, height, weight, body fat, menstrual status, sporting group and original $\dot{V}O_2$max was investigated. Linear models (McCullagh and Nelder, 1983; GLIM, 1986) were used to assess which of these 7 parameters, or combination of them, could best explain the variation in bone mineral density at each of the 3 sites around the hip ("Statistical Methods", chapter 7.2).

10.3.3 RESULTS

Age, height, weight and body fat for each amenorrhoeic and oligomenorrhoeic athlete is given in table 10.3.1 and for each normal oestrogen status athlete in table 10.3.2. The mean (95% confidence interval) values for each of these variables in each of the menstrual groups is given in table 10.3.3.

The bone mineral content (BMC) of the femoral neck, Ward's triangle and trochanteric regions of the left hip for each athlete are given in tables 10.3.4 and 10.3.5. Table 10.3.6 gives the mean (95% confidence interval) values for the BMC at each of these 3 sites in each of the 4 menstrual groups. The BMC in the trochanteric region was significantly lower (P= 0.010) in the amenorrhoeic athletes (0.66 gm/cm^2) than in the other 3 groups (0.75 gm/cm^2 in the oligomenorrhoeics and oral contraceptive takers and 0.78 gm/cm^2 in the eumenorrhoeics) - figure 10.3.1. There were no significant differences in the BMC between the menstrual groups at either Wards triangle (P= 0.34) or the femoral neck (P= 0.18) - figures 10.3.2 and 10.3.3.

Table 10.3.7 gives the mean (95% confidence interval) values for the BMC at each of the 3 hip sites in each of the 3 sporting groups. There were no significant differences between the sporting groups at any of these sites (P= 0.26 at the trochanteric region; P= 0.40 at Ward's triangle; P= 0.96 at the femoral neck).

Table 10.3.8 gives the results of model building for the trochanteric region. In addition to menstrual status (P= 0.0098), weight (P= 0.0023) and body mass index (P= 0.0088) were very significantly related to the BMC (stage 1). However once weight was incorporated into the model (stage 2), no other variable had a significant effect on BMC.

Tables 10.3.9 and 10.3.10 give the results of model building for Ward's triangle and the femoral neck respectively. Age was very significantly related (P= 0.0070) to BMC at Ward's and marginally related (P= 0.055) at the femoral neck (stage 1 in each table). Once this was incorporated into each of the models no other variable exerted a significant effect (stage 2 in each table).

10.3.4 DISCUSSION

Most of the previous data on the bone density changes in female athletes have focused on the lumbar spine and the forearm. These have tended to show significant bone density reductions in the spine in amenorrhoeic athletes and, in some cases, marginal reductions at trabecular sites in the forearm (Drinkwater et al, 1984; Lindberg et al, 1984; Marcus et al, 1985).

Using the Hologic dual energy x-ray absorptiometer, we have been able to get accurate bone density measurements at 3 sites around the left proximal femur. The BMC in the femoral neck region, which consists of cortical bone predominantly, was similar in all

the menstrual groups. The bone density of Ward's triangle was also similar in all the menstrual groups. However young adults may not have a defined area of minimum density in the femoral neck and so analysis may not give a true density for Ward's triangle (Hologic QDR-1000™ Operator's manual).

Nevertheless bone density at each of these 2 sites was, to a greater or lesser extent, positively related to age. The mean age of our study group was 25.8 years with a range of 19-32 which suggests that bone mass in the hip continues to rise throughout the twenties. By contrast Stevenson et al (1989), who studied a group of 112 premenopausal women, showed significant negative correlations between age and bone density at the femoral neck, Ward's triangle and the trochanteric region (the relationship was stronger at the former 2 sites). However the mean age (34.8 yrs) and range (21-52 yrs) were higher in their study. Taken together these 2 studies suggest that peak bone mass at the hip may occur around the age of 30 years.

In the trochanteric region, where there is a larger proportion of trabecular bone, we were able to demonstrate significant differences between the menstrual groups. As in the lumbar spine, the bone density at this site was significantly lower in the amenorrhoeics than the other menstrual groups. In fact, trochanteric and lumbar spine bone density are strongly correlated (see chapter 10.4).

Despite having shown higher bone density levels in the mid-shaft of the femur in the runners, we were unable to show differences in any of the 3 proximal femur sites between the 3 sporting groups. This is somewhat surprising in view of the fact that these sites are only separated by about 20 - 25 cm and suggests that the anabolic stimulus produced by intense running is not sufficient to reach as far as the hip. An alternative hypothesis is based on the fact that during load-bearing (eg running) the greatest transverse movement in a long bone will take place at its mid-point with very little at either end. This movement, with associated stretching of the periostium, may act as an anabolic stimulus to the bone and be responsible for the increased bone density at the mid-point.

The reduced spinal bone density levels in amenorrhoeic athletes has raised concern regarding the risk of vertebral crush fractures. That we have also shown reduced bone density levels at 1 of 3 sites in the hip must also cause concern regarding the risk of fracture at this site.

10.3.5 CONCLUSIONS

1. The bone density of the femoral neck and Ward's triangle was not affected by menstrual status but did increase with age throughout the twenties.

2. The bone density of the trochanteric region was reduced in amenorrhoeic athletes.

3. The bone density at all 3 sites in the hip was not affected by sporting activity group.

TABLE 10.3.1

ANTHROPOMETRIC PARAMETERS IN THE AMENORRHOEIC AND OLIGOMENORRHOEIC ATHLETES AT THEIR SECOND VISIT
(A= amenorrhoeic; O= oligomenorrhoeic)
(Da= dancer; Ro= rower; Ru= runner)

REG.NO.	MEN.STAT	SPORT	AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)	BODY FAT (%)
007	A	Ro	27	168	59	16.8
008	A	Ro	31	166	73	27.9
023	A	Da	21	161	44	16
024	A	Ro	21	174	56	20
026	A	Da	23	165	48	14.6
031	A	Da	19	171	61	23.2
042	A	Da	22	165	51	19.7
043	A	Ru	27	159	52	16.4
046	A	Ru	28	163	48	15.9
052	A	Ru	26	156	46	14.9
060	A	Ru	21	166	55	20.9
063	A	Ru	28	162	48	13.1
064	A	Ru	28	164	48	10.9
066	A	Ru	23	160	49	20.5
068	A	Ru	29	155	48	19.7
002	O	Ro	24	183	75	19.1
011	O	Ru	27	165	59	16.3
014	O	Ro	23	173	62	20.8
025	O	Ro	21	171	63	21.9
028	O	Ro	22	163	62	18.8
048	O	Ru	30	163	54	19.7
054	O	Ru	24	168	62	22.2
061	O	Ro	21	162	63	25.5

TABLE 10.3.2

ANTHROPOMETRIC PARAMETERS IN THE NORMAL OESTROGEN STATUS ATHLETES AT THEIR SECOND VISIT
(E= eumenorrhoeic; C= oral contraceptive)
(Da= dancer; Ro= rower; Ru= runner)

REG.NO.	MEN.STAT	SPORT	AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)	BODY FAT (%)
004	E	Ro	26	176	73	21
006	E	Ro	31	168	62	24.3
009	E	Ro	21	174	71	22.5
017	E	Ro	27	168	57	15.7
019	E	Ro	24	170	65	19.8
020	E	Ro	32	171	66	18.3
021	E	Ro	25	184	75	20.1
027	E	Ro	23	175	67	20.1
032	E	Ro	26	159	56	16.8
033	E	Ro	25	173	63	22
040	E	Da	25	167	50	17.5
047	E	Ru	28	163	50	19.8
051	E	Ru	31	165	51	17.1
055	E	Da	31	160	50	24.8
056	E	Da	21	164	58	18.8
058	E	Ro	30	169	61	15.7
059	E	Ru	26	172	61	20.8
062	E	Ru	26	170	58	21.7
065	E	Ru	29	164	51	21.6
070	E	Ru	26	167	59	15.8
001	C	Ro	23	183	80	19.7
003	C	Ro	26	165	67	23.6
005	C	Ro	24	170	60	15.6
010	C	Ro	29	170	63	19
012	C	Ro	26	175	65	17.7
015	C	Ro	32	174	69	20.8
016	C	Ro	26	180	77	18.2
018	C	Ro	27	169	72	23.2
022	C	Ro	28	167	61	22.7
030	C	Ro	22	159	53	17.3
034	C	Ro	26	169	60	21.8
035	C	Ro	27	167	59	22.8
037	C	Da	28	166	50	18.3
044	C	Ro	24	181	74	16.4
049	C	Ru	25	175	58	16.9
050	C	Ru	30	164	50	18.9
053	C	Ru	22	160	48	15.8
057	C	Da	25	161	48	16.9

TABLE: 10.3.3 Mean (95% Confidence Interval) Background Variables in the 4 Menstrual Groups

	Amenorrhoea	Oligomen.	Eumen.	Oral Contraceptive	ANOVA
Number	15	8	20	18	
Age (Yrs)	24.9 (23.3,26.6)	24.0 (21.7,26.3)	26.7 (25.2,28.1)	26.1 (24.6,27.6)	**F=1.74 P=0.17**
Height (cm)	163 (160,167)	169 (164,173)	169 (166,172)	170 (167,173)	**F=3.00 P=0.038**
Weight (kg)	52.4 (48.2,56.6)	62.5 (56.7,68.3)	60.2 (56.5,63.9)	61.9 (58.0,65.6)	**F=4.61 P=0.006**
Body Fat (%)	18.0 (16.4,19.7)	20.5 (18.3,22.8)	19.7 (18.3,21.1)	19.2 (17.7,20.7)	**F=1.32 P=0.277**

TABLE 10.3.4

BONE DENSITY OF THE LEFT PROXIMAL FEMUR IN THE AMENORRHOEIC AND OLIGOMENORRHOEIC ATHLETES
(A= amenorrhoeic; O= oligomenorrhoeic)

REG.NO.	MEN.STAT	LEFT HIP BONE DENSITY (gm/cm^2)		
		Neck	Ward's	Trochanteric
007	A	.948	.877	.785
008	A	.726	.622	.641
023	A	.850	.695	.613
024	A	.797	.686	.694
026	A	.635	.476	.556
031	A	.961	.878	.700
042	A	.969	.870	.691
043	A	1.066	.925	.889
046	A	.748	.464	.483
052	A	.738	.625	.630
060	A	1.055	.815	.824
063	A	.754	.616	.532
064	A	.810	.614	.563
066	A	.785	.567	.567
068	A	.962	.774	.735
002	O	.799	.633	.671
011	O	.791	.612	.609
014	O	.849	.666	.728
025	O	.946	.739	.886
028	O	.961	.900	.893
048	O	.892	.608	.641
054	O	1.035	.794	.741
061	O	1.209	1.039	.862

TABLE 10.3.5

BONE DENSITY OF THE LEFT PROXIMAL FEMUR IN THE NORMAL OESTROGEN STATUS ATHLETES
(E= eumenorrhoeic; C= oral contraceptive)

REG.NO.	MEN.STAT	LEFT HIP BONE DENSITY (gm/cm^2)		
		Neck	Ward's	Trochanteric
004	E	.850	.666	.741
006	E	.706	.560	.622
009	E	.884	.823	.825
017	E	.953	.801	.846
019	E	.958	.816	.802
020	E	.848	.660	.853
021	E	1.044	.878	.768
027	E	.814	.627	.717
032	E	.846	.688	.715
033	E	1.077	.860	.742
040	E	1.096	.838	.855
047	E	.863	.648	.639
051	E	.945	.801	.920
055	E	.859	.726	.682
056	E	.879	.869	.788
058	E	.915	.790	.823
059	E	.922	.736	.933
062	E	.924	.781	.673
065	E	1.076	.855	.771
070	E	.922	.810	.860
001	C	.914	.825	.871
003	C	.945	.702	.682
005	C	.836	.676	.714
010	C	.861	.708	.777
012	C	.908	.695	.844
015	C	.865	.656	.793
016	C	.911	.799	.852
018	C	.863	.710	.720
022	C	.849	.643	.657
030	C	.927	.770	.798
034	C	.862	.746	.733
035	C	.778	.577	.577
037	C	.910	.717	.788
044	C	.956	.709	.882
049	C	.949	.753	.809
050	C	.724	.551	.519
053	C	.831	.652	.802
057	C	.922	.852	.686

TABLE 10.3.6: Mean (95% Confidence Interval) Proximal Femur Bone Density in the Menstrual Groups

Menstrual Groups	N	WARDS (gm/cm^2)	NECK (gm/cm^2)	TROCHANTERIC (gm/cm^2)
Amen	15	0.70 (0.64,0.76)	0.85 (0.80,0.91)	0.66 (0.61,0.71)
Oligomen	8	0.75 (0.67,0.83)	0.94 (0.86,1.01)	0.75 (0.68,0.83)
Eumen	20	0.76 (0.71,0.81)	0.92 (0.87,0.97)	0.78 (0.73,0.82)
Oral Contracep.	18	0.71 (0.65,0.76)	0.88 (0.83,0.93)	0.75 (0.70,0.80)
---	---	---	---	---
ANOVA	$F_{3,57}$	1.15	1.69	4.16
	P	0.34	0.18	0.010

TABLE 10.3.7 : Mean (95% Confidence Interval) Proximal Femur Bone Densities in the Sporting Groups.

Sport	N	Wards (gm/cm^2	Neck (gm/cm^2)	Trochanteric (gm/cm^2)
Dance	9	0.77 (0.69,0.85)	0.90 (0.83,0.97)	0.71 (0.63,0.78)
Row	33	0.73 (0.69,0.77)	0.89 (0.85,0.93)	0.76 (0.72,0.79)
Run	19	0.70 (0.65,0.76)	0.89 (0.85,0.94)	0.71 (0.66,0.76)
---	---	---	---	---
ANOVA	$F_{2,58}$	0.94	0.04	1.37
	P	0.40	0.96	0.26

TABLE: 10.3.8 Model Fitting : TROCHANTERIC REGION

VARIABLE	STAGE 1 (P-value)	STAGE 2 (P-value)
Men. Stat.	0.0098	0.095
Sport	0.19	0.84
Age	0.19	0.23
Height	0.011	0.86
Weight	**0.0023**	-
BMI	0.0088	1.00
Calcium Intake	0.73	0.33
Body Fat	0.74	0.28
$\dot{V}O_2$ **max**	0.16	0.36

Stage 1:
Values in the column represent the probability from the significance test of the relationship between each single variable and the bone density of the Trochanteric region. The one with the smallest P-value is incorporated into the model.

Stage 2:
Values in the column represent the probability from the significance test of any additional relationship between each remaining variable and the bone density of the Trochanteric region.

TABLE 10.3.9 : Model Fitting : WARDS TRIANGLE

VARIABLE	STAGE 1 (P-value)	STAGE 2 (P-value)
Men. Stat.	0.34	0.12
Sport	0.38	0.76
Age	**0.0070**	-
Height	0.54	0.79
Weight	0.19	0.23
BMI	0.12	0.09
Calcium Intake	0.16	0.12
Body Fat	0.21	0.19
$\dot{V}O_2$ **max**	0.049	0.12

Stage 1:
Values in the column represent the probability from the significance test of the relationship between each single variable and the bone density of Ward's Triangle. The one with the smallest P-value is incorporated into the model.

Stage 2:
Values in the column represent the probability from the significance test of any additional relationship between each remaining variable and the bone density of Ward's Triangle.

TABLE 10.3.10 : Model Fitting : FEMORAL NECK

VARIABLE	STAGE 1 (P-value)	STAGE 2 (P-value)
Men. stat	0.18	0.11
Sport	0.98	0.87
Age	**0.055**	-
Height	0.50	0.68
Weight	0.35	0.40
BMI	0.32	0.29
Calcium Intake	0.16	0.14
Body Fat	0.15	0.15
$\dot{V}O_2$ max	0.14	0.25

Stage 1:
Values in the column represent the probability from the significance test of the relationship between each single variable and the bone density of the femoral neck. The one with the smallest P-value is incorporated into the model.

Stage 2:
Values in the column represent the probability from the significance test of any additional relationship between each remaining variable and the bone density of the femoral neck.

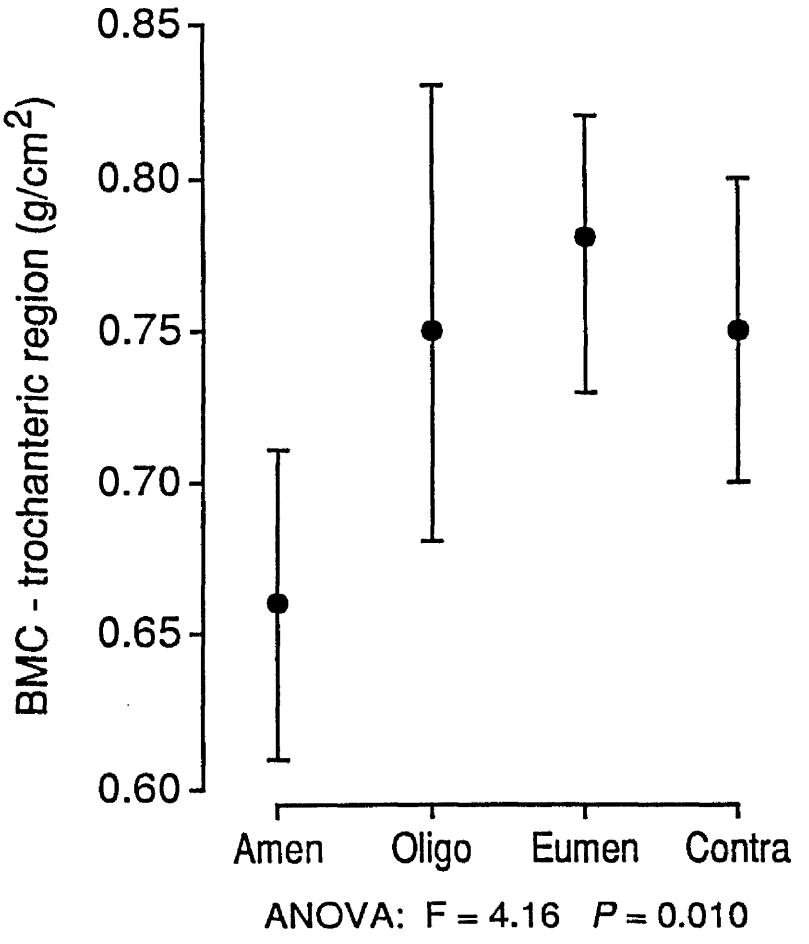

FIGURE 10.3.1:

The Bone Mineral Content of the Trochanteric region (mean with 95% confidence interval) in the Amenorrhoeic, Oligomenorrhoeic, Eumenorrhoeic and Oral Contraceptive-taking Athletes.

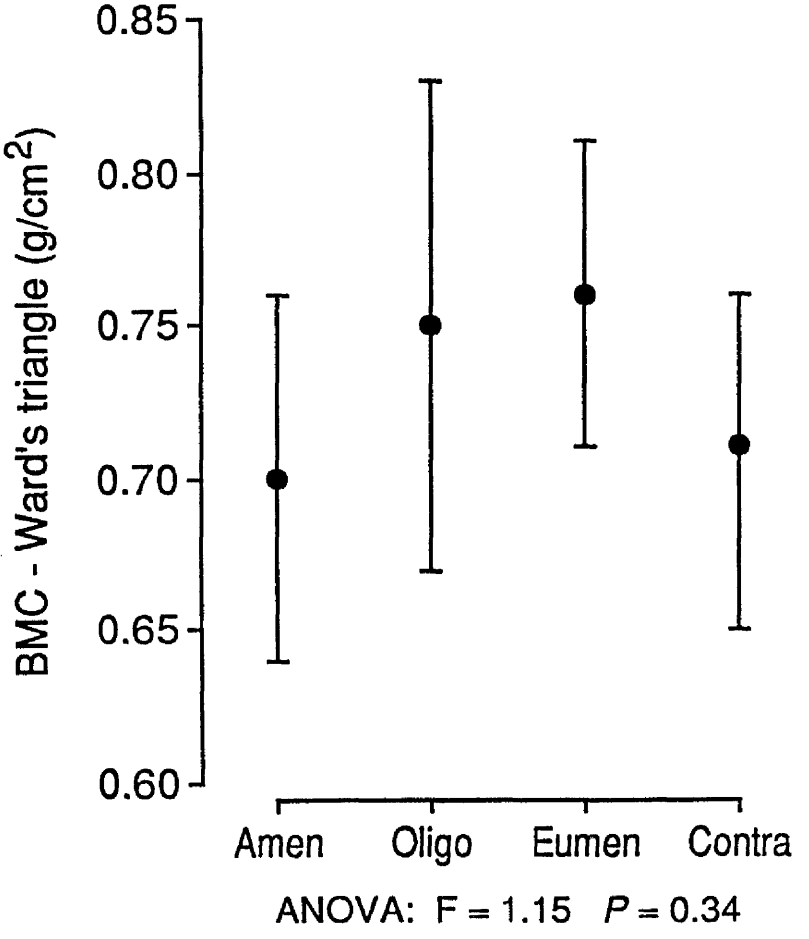

FIGURE 10.3.2:

The Bone Mineral Content of Ward's Triangle (mean with 95% confidence interval) in the Amenorrhoeic, Oligomenorrhoeic, Eumenorrhoeic and Oral Contraceptive-taking Athletes.

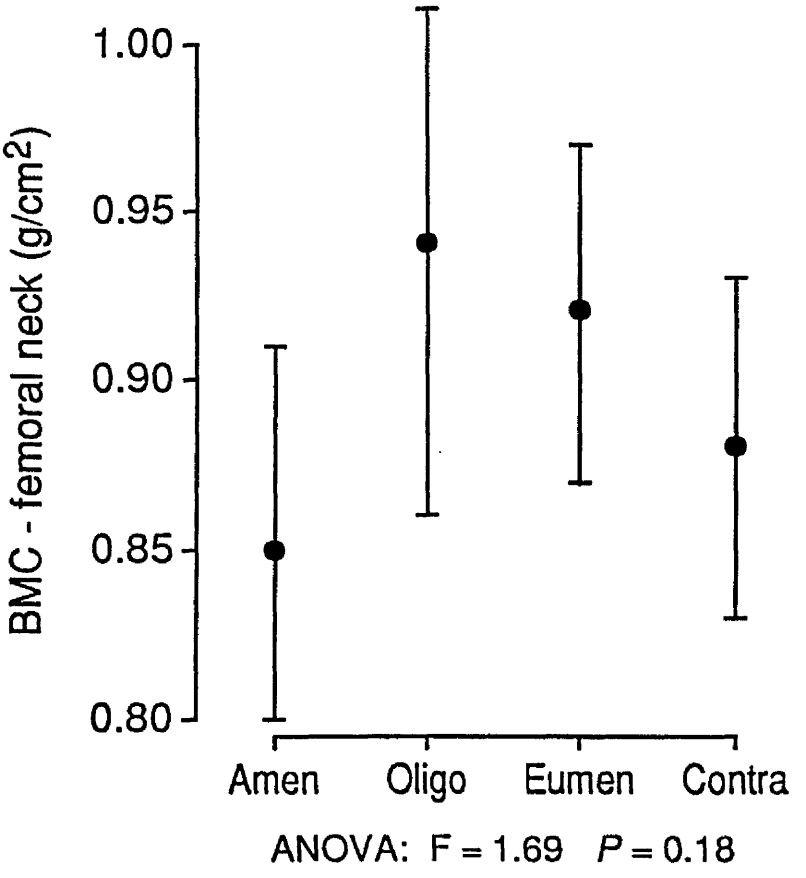

Figure 10.3.3:

The Bone Mineral Content of the Femoral Neck (mean with 95% confidence interval) in the Amenorrhoeic, Oligomenorrhoeic, Eumenorrhoeic and Oral Contraceptive-taking Athletes.

10.4 RELATIONSHIP BETWEEN BONE DENSITY AT DIFFERENT SITES

10.4.1 INTRODUCTION

We have measured the bone density at 5 different sites in the skeleton. The spinal measurement consists predominantly of trabecular bone, the femoral midshaft measurement of cortical bone and the 3 hip measurements (trochanteric, Ward's and neck) consist of a varying proportion of the 2 types of bone. We have investigated the relationship between the bone density at each of the 5 sites.

10.4.2 METHOD

The bone density was measured at each of 5 sites in 62 athletes (chapter 10.1). These were :-

a) lumbar spine
b) femoral midshaft
c) femoral neck
d) Ward's triangle and
e) trochanteric region

The methods of measurement are described in chapter 3.4. For each athlete the bone density evaluations at each of the sites was performed on the same day.

The relationship between the bone density at each of the sites was investigated using Pearson product moment correlation coefficients.

10.4.3 RESULTS

The spinal bone density measurements from the second attendance are given in tables 10.2.1, 10.2.3, 10.2.5 and 10.2.7. The femoral shaft measurements are given in tables 10.2.2, 10.2.4, 10.2.6 and 10.2.8. The 3 hip bone density measurements are given in tables 10.3.4 and 10.3.5. In addition, measurements were taken from subject number 036, who had been pregnant during the year (chapter 10.1). Her measurements were as follows:-

Spinal trabecular bone density	187	mg/cm^3
Femoral midshaft	1.21	g/cm^2
Femoral neck	0.805	g/cm^2
Ward's triangle	0.551	g/cm^2
Trochanteric region	0.593	g/cm^2.

The relationship between the bone densities at each of the 5 sites is given in table 10.4.1. The 3 hip bone density measurements were highly correlated with each other ($P< 0.0001$) in all 3). Spinal bone density was very significantly related to trochanteric density ($P< 0.0001$), significantly related to Ward's ($P= 0.012$) and femoral neck ($P= 0.016$) and not related to femoral shaft (figure 10.4.1). Femoral shaft bone density was significantly related to the 3 hip bone density measurements.

10.4.4 DISCUSSION

Previous research has shown bone density measurements at various sites to be highly correlated. Seldin et al (1988) showed that femoral neck, Ward's triangle and trochanteric bone density measurements were very closely related to each other and that spinal density was closely related to all these hip measurements. Dual photon absorptiometry was used at all sites. The correlation between these 4 sites and the bone density in the wrist evaluated using single photon absorptiometry was statistically significant but less strong. Schaadt and Bohr (1988), also using dual photon absorptiometry, showed very high correlations between the lumbar spine, femoral neck and femoral shaft measurements.

We have used 3 different methods for bone density evaluation at each of the 3 anatomical regions. The methods of measurement at the femoral shaft and proximal femur are similar and are expressed in the same units (g/cm^2). High correlations between the evaluations at these sites are therefore expected.

However spinal bone density was evaluated by a totally different method, is expressed in different units (mg/cm^3) and provides a measure predominantly of trabecular bone - see chapter 3.4. The lack of relationship with femoral shaft, which consists predominantly of cortical bone, is not surprising.

The relationships between spinal bone density and the 3 hip measurements were significant even though the methods of measurement were different. However the correlation with the trochanteric region was much stronger than with the other 2 hip sites. Furthermore the correlation between the femoral neck and Ward's triangle was stronger than between either of these and the trochanteric region. This suggests that the latter hip site behaves differently from the former 2. This is probably due to the high content of trabecular bone around the greater trochanter whereas the femoral neck region contains a higher proportion of cortical bone (Bohr and Schaadt, 1985).

10.4.5 CONCLUSIONS

1. Bone density measurements at the 3 anatomical sites tend to be highly correlated.

2. Trabecular bone density in the spine is most closely correlated to the trochanteric region of the hip.

3. Spinal trabecular bone density and cortical bone density of the femoral midshaft are not significantly correlated.

TABLE 10.4.1 : Correlation Co-efficients (P-value) between the Bone Densities at the Five Sites.

	Femoral Shaft	Neck	Wards	Trochanteric
Lumbar Spine	0.18 (0.17)	0.30 (0.016)	0.32 (0.012)	0.60 (<0.0001)
Femoral Shaft	-	0.46 (0.0002)	0.49 (<0.0001)	0.47 (0.0001)
Neck	-	-	0.87 (<0.0001)	0.67 (<0.0001)
Ward's	-	-	-	0.70 (<0.0001)

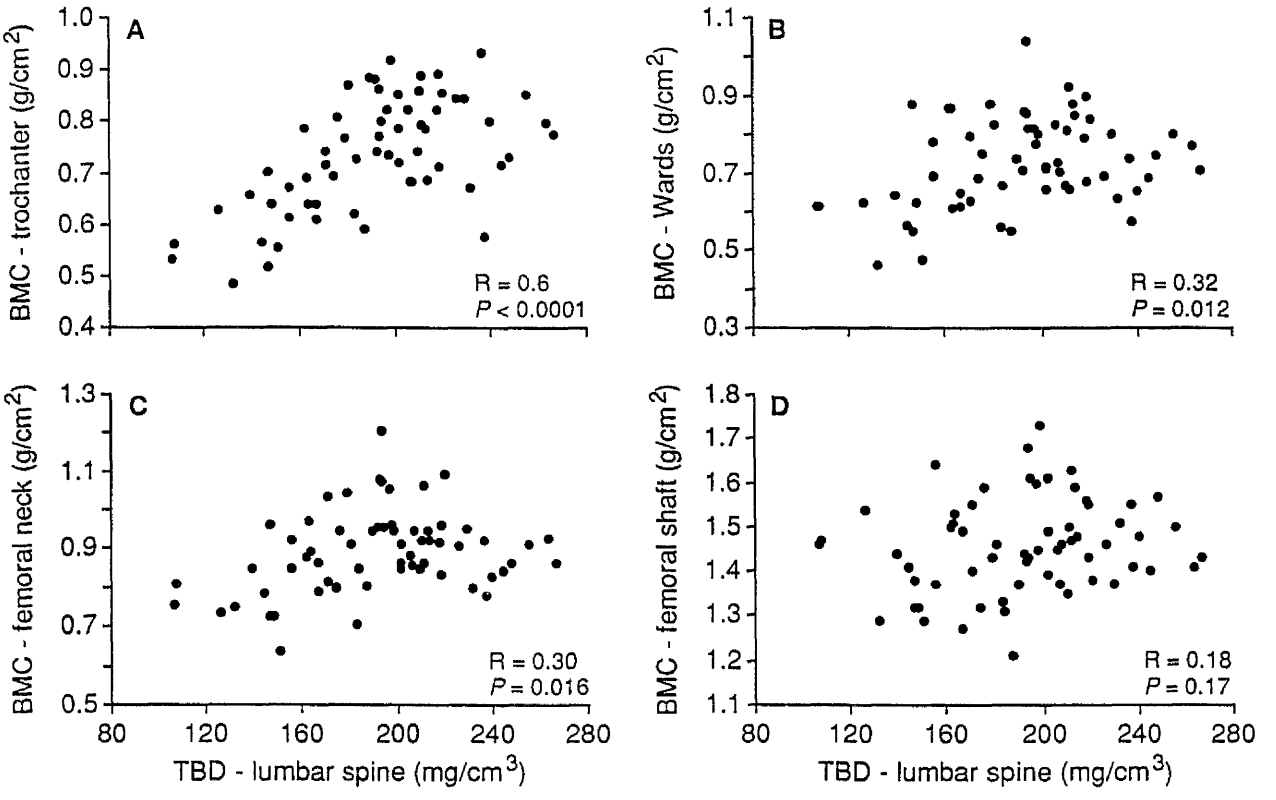

Figure 10.4.1:

Correlation between Spinal TBD and the Bone Density of the Trochanteric Region (A), Ward's Triangle (B), the Femoral Neck (C) and the Femoral Shaft (D).

CHAPTER 11: GENERAL CONCLUSIONS AND FUTURE PROSPECTS

This thesis has been concerned with the effect of 3 factors, namely physical activity, oestrogen status and calcium intake, on bone density levels in elite female athletes. This chapter discusses the relevance of this work to current knowledge and possible future areas of research.

11.1 PHYSICAL ACTIVITY

General Conclusions

We have demonstrated the benefits of certain broad categories of exercise on different parts of the skeleton. Rowing, by virtue of intensive trunk exercise, appeared to enhance the bone density in the spine (chapter 6.1) compared not only to sedentary controls but also to athletes doing other sports. Furthermore rowing may partly avert the spinal bone density losses due to amenorrhoea. Running, a weight bearing activity, is associated with an enhanced bone density of the femoral midshaft (chapter 6.2) compared to athletes in other sports. These findings are consistent with a localized anabolic response of the skeleton to exercise, a point dramatically illustrated in tennis players (chapter 2.2) where the bone density in the playing arm can be

30% higher than on the other side. They also demonstrate that weight-bearing exercise is likely to be important in providing an anabolic stimulus to bone (chapter 2.2.2).

Additionally we have shown a complex relationship between aerobic capacity and bone density in the spine (chapter 7). In "normal" oestrogen status athletes this is linear and positive, but at very high levels of $\dot{V}O_2max$ where there is associated amenorrhoea and low levels of body fat the apparent benefits are lost. Previous work has investigated this relationship in women with much lower levels for $\dot{V}O_2max$ and has shown that, at best, aerobic capacity is only a weak predictor of bone density.

We have been unable to demonstrate any convincing statistical relationship between muscle strength and bone density. Other studies have revealed conflicting information on this relationship (reviewed in chapter 7.4) but this may be due to the many different methods of measuring muscle strength. Isokinetic strength, the system we have used, is a more physiological method of assessment. However isokinetic testing of the back involves movement at several different points in the spine and may not be representative of forces placed on the second, third and fourth lumbar vertebrae, the sites where we measured bone density. Isokinetic strength measured at a site where movement occurs around a single point may be more closely related to the bone density in the adjacent skeleton (see below).

Future Prospects

The type, intensity and duration of exercise that provides the maximum anabolic stimulus to bone has not been clearly defined. We have shown that in young adults, the bone density can be 10-20% higher in normal oestrogen status athletes compared to sedentary controls (chapter 5) and this is likely to enhance the peak bone mass at skeletal maturity (see below). Studying athletes from different sports and identifying those training habits which are associated with anabolic effects on bone, could be helpful in developing exercise strategies for reducing the incidence of osteoporotic fractures if peak bone mass is shown to be an important determinant of fracture risk in later life.

In older adults, including postmenopausal women, exercise can also enhance bone mass (chapter 2.2.2). There is, for example, some evidence that even walking may enhance bone density in older populations (Zylstra et al, 1989). However intervention studies investigating the effect of different exercise patterns on bone density in this age-group are needed.

Our understanding of the effect of exercise on the skeleton may be enhanced by investigating its relationship with bone turnover. Although this thesis demonstrated only weak correlations between exercise and biochemical indices of bone turnover measured cross-sectionally, longitudinal studies using more specific measures of bone formation (eg skeletal isoenzyme of alkaline phosphatase - Taylor et al, 1987) and resorption (eg urinary

pyridinium crosslinks and tartrate-resistant acid phosphatase - Azria, 1989) may provide more fruitful results. Certainly studies on the changes in serum osteocalcin with exercise (reviewed in chapter 9.4) have been encouraging.

The relationship between isokinetic muscle strength (at different speeds) and bone density at adjacent sites requires further investigation. For example, isokinetic muscle strength at the knee and bone density of different sites in the femur may be anticipated to reveal significant correlations.

11.2 OESTROGEN STATUS

General Conclusions

We have shown that trabecular bone density in the spine is reduced in low oestrogen status athletes, but that cortical bone in the femoral midshaft is not. Previous studies have also demonstrated reductions in spinal trabecular bone and, in addition, in trabecular, but not cortical, bone of the forearm. The lack of effect seen in cortical bone may be due to its low turnover rate compared to trabecular bone, so that several years of amenorrhoea are necessary before detectable changes occur. The reduction in trochanteric bone density of the hip (chapter 10.3),

a site that contains trabecular bone, in amenorrhoeic athletes has not previously been reported and may increase the risk of hip fracture in later life.

We have also found evidence for high bone turnover rates in amenorrhoeic athletes (chapter 9), a feature seen in amenorrhoeic women on LHRH agonists (Johansen et al, 1988) and in normal women after the menopause. This suggests that in low oestrogen status women there is increased bone resorption with an associated increase in formation. Whether this is a direct or indirect effect of low oestrogen status on the skeleton remains uncertain (chapter 8.4).

There is very little information on the effect of the modern, low dose oestrogen-containing oral contraceptive on bone density. We have investigated this relationship and found that bone density in the spine, femoral shaft and hip is similar to the level seen in eumenorrhoeic athletes. In some of our analyses we have put these 2 groups together and classified them as "normal" oestrogen status. However they may not be a totally homogeneous group as bone turnover rates seem to be lower in the oral contraceptive-takers (chapter 9).

Future Prospects

Longitudinal studies can provide information on the effect of changing oestrogen status and, in particular, whether bone density will improve once eumenorrhoea is re-established. Unfortunately only very few amenorrhoeic athletes regained their periods during the course of the study (chapter 10.2) and we were therefore unable to determine the effect of resumption of menses on bone density. We were also unable to demonstrate any effect on bone density of changes in exercise intensity and calcium intake which suggests that the time length of follow up may not have been sufficiently long to demonstrate any such trends. Previous studies have suggested that when menstruation returns the bone density, at least, partially recovers (reviewed in chapter 10.2.4). Further longitudinal studies (with follow-up for several years) are needed to determine how bone density changes with very prolonged episodes of amenorrhoea and whether these changes eventually become irreversible.

11.3 AMENORRHOEIC ATHLETES.

General Conclusions

We have identified the sports in which there is a high incidence of amenorrhoea (chapter 4). These include ballet, cycling, running, rowing (light weight) and gymnastics.

The oestrogen status of the gymnasts, who usually retire before the end of their teenage years, will tend to be normal by the age of twenty and they are therefore unlikely to be at risk from osteoporosis. The rowers, by virtue of their exercise, will tend to have less severe reductions of bone density in the spine, the site most severely affected by low oestrogen status.

The most severe reductions in spinal bone density occur in the runners and dancers, especially in those with prolonged episodes of amenorrhoea. Both sports predominantly involve lower body exercise with only moderate exercise stimulation to the spine. The ones with very high levels of $\dot{V}O_2max$ will have associated low levels of body fat and low peripheral oestrogen production (chapter 7). These athletes, which include the top class runners, may have marked reductions in spinal bone density (non-linear relationship between bone density and $\dot{V}O_2max$ - chapter 7). The other factor that may also be highly relevant to athletes in these 2 sports is that of relative nutritional (including calorie) deficiency - see below.

Future Prospects

A high incidence of amenorrhoea is also seen in cycling, a sport which involves lower body exercise and only partial weight-bearing. Although our research has not included athletes from this sport, we would predict that cyclists with prolonged amenorrhoea may well have marked reductions in spinal bone density. Further research on cyclists would therefore be valuable.

Amenorrhoeic athletes could perhaps be at greater risk of developing osteoporosis in later life. Studies of ex-athletes, who had episodes of amenorrhoea when training (such as runners), may be useful in determining this risk.

We have shown that athletes on the oestrogen-containing oral contraceptive, including some who may otherwise have been amenorrhoeic, have similar bone density levels to their eumenorrhoeic counterparts (chapter 5). This suggests that oestrogen replacement either in the form of the oral contraceptive or hormone replacement therapy may be an effective treatment for amenorrhoeic athletes. However there are no published data on its effect in this group and intervention studies are needed. Studies on the use of Calcium supplementation in enhancing bone mass in this group are also required (see below).

11.4 NUTRITION

General Conclusions and Future Prospects

Some amenorrhoeic athletes develop nutritional deficiencies. Many on calorie restricted diets become calorie deficient (Nelson et al, 1986). They may also be on diets deficient in calcium, vitamins, zinc (Deuster et al; Fertil Steril; 1986), iron (Deuster et al; Am J Clin Nutr; 1986) and other trace elements. Such deficiencies might also have an adverse effect on bone density and the attainment of peak bone mass (Parfitt, 1983), although this has not been clearly defined.

We have demonstrated that increased dietary calcium intake is associated with increased spinal bone density in women in their twenties (chapter 8). We have been unable to determine whether this is a direct or indirect effect of calcium (eg calcium intake is closely related to calorie and energy intake) and therefore it remains uncertain whether calcium supplementation would be useful in young, female adults. A 2 year prospective study investigating the effect of calcium supplementation on spinal bone density and controlling both for calorie intake and exercise type and intensity may help to resolve this issue.

11.5 INTERACTION OF EXERCISE, OESTROGEN STATUS AND DIET

General Conclusions

This thesis has investigated the consequence of extreme exercise on the skeleton. Those athletes who maintain normal oestrogen status while performing intense exercise can enhance their bone density. Spinal bone density can be 10-20% higher than in eumenorrhoeic, sedentary women of a similar age (chapter 5). Paradoxically such high levels of endurance exercise can also lead to disturbance of hypothalamic-pituitary-ovarian axis function, which negates the direct benefits of exercise on the skeleton. In such circumstances trabecular bone density can fall, on average, by 12% but in some individuals the loss can be much greater.

This link between endurance exercise, oestrogen status and the skeleton is well demonstrated by the relationship seen between $\dot{V}O_2max$ and trabecular bone density (chapter 7). Up to $\dot{V}O_2max$ levels of about 60 mls/kg/min there is a steady rise in bone density, but with aerobic fitness above this point oestrogen production and release are impaired and bone density falls.

Future Prospects

Nutrition will also influence bone density. In many athletes, calorie restriction forms an important component of training but will impair hypothalamic-pituitary-ovarian axis function (chapter 2.1) leading indirectly to a negative effect on the skeleton. The interaction of calorie intake, calcium intake and energy expenditure and its relationship to bone density is not fully understood and requires further study.

PEAK BONE MASS

Bone loss in the early postmenopausal period is almost universal but it may occur at different rates in individual women. Nevertheless, the time it takes to reach the so-called fracture threshold will partly be determined by the peak bone mass which is usually achieved in the early thirties (figure 11.1). Oestrogen status, physical activity and calcium are all important determinants of peak bone mass but there are probably genetic limitations on it as well.

We have shown that a combination of normal oestrogen status, high levels of physical activity and good calcium intake in the diet during the twenties are associated with increased peak bone mass and this may reduce the risk of osteoporosis in later life. Previous work on both amenorrhoeic athletes and immobilized

patients suggests that deficiency of one factor cannot generally be compensated for by extremes of the other 2 and in such circumstances the risk of bone loss is probably increased. Our work however indicates that specific types of exercise and/or high intakes of calcium may partly offset the losses sustained by low oestrogen status. Whether this applies just to young women in their twenties, or also to women in the peri- and post-menopausal period remains uncertain and should form the basis of further research.

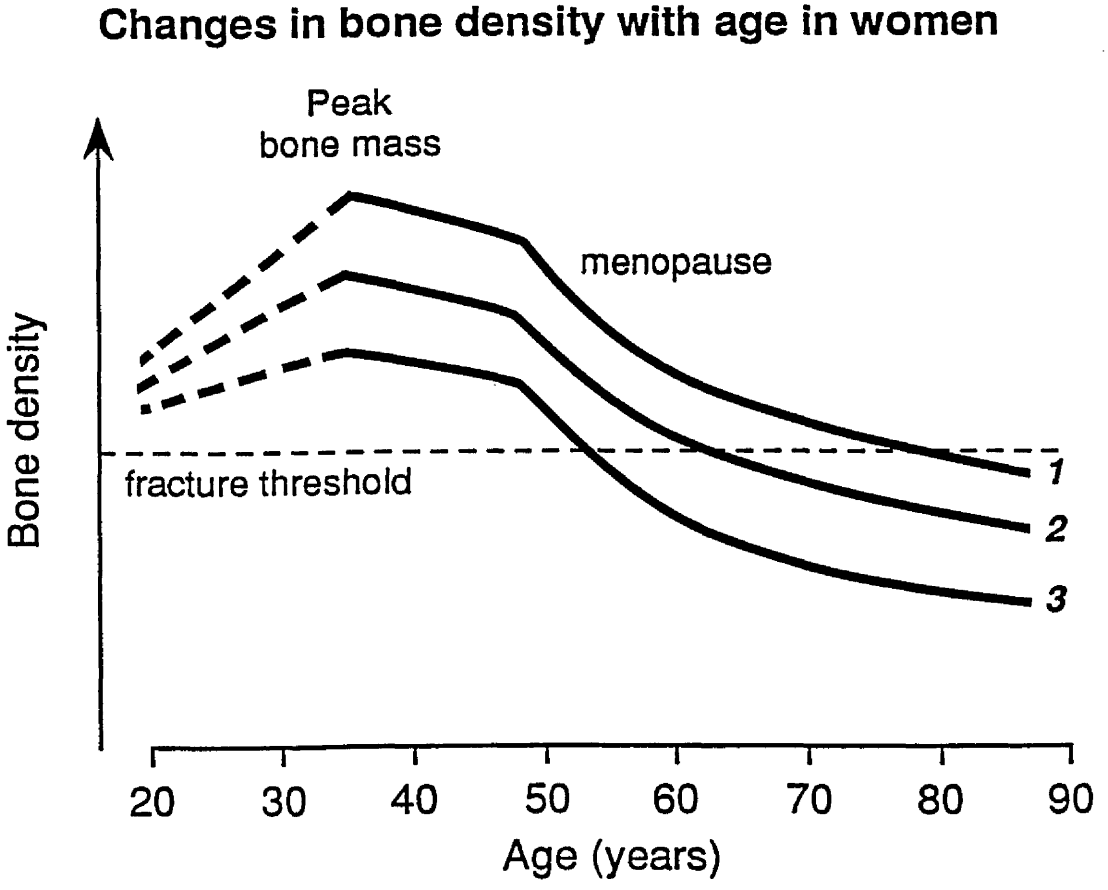

Figure 11.1:

Theoretical Changes in Bone Density with Age in Eumenorrhoeic, Sedentary Women (2), Eumenorrhoeic Athletes (1), Amenorrhoeic Athletes (3).

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APPENDIX

ADDENDUM: METHODOLOGICAL CONSIDERATIONS IN THE USE OF BONE DENSITOMETRY

Further consideration of the follow-up data showed considerable variation in spinal trabecular bone density (tables 10.2.1, 10.2.3, 10.2.5 and 10.2.7) compared to changes seen in the midshaft of the femur (tables 10.2.2, 10.2.4, 10.2.6 and 10.2.8). These observed changes did not differ significantly between the menstrual status groups (table 10.2.9). Until recently there has been no relevant comparative data. However a study by Prior et al (1990), published shortly after this thesis was submitted, investigated spinal trabecular bone density changes over a one year period in a group of female marathon runners. Their data differed significantly from our own and, although the subjects from the 2 studies are not well matched (see below), this has allowed us to analyse critically our own spinal bone density data and consider possible methodological and biological variables which might have had a bearing on our results.

QCT METHODOLOGY

The methodology for QCT has been described briefly in chapter 3.4.3. Several points require further clarification.

A General Electric CT 9000 scanner was used to measure spinal bone density. This was situated in a General Radiology department. Bone density measurements took place almost every day between 9.00 and 9.30 am and were supervised by one of three senior radiographers. The scanner was calibrated at the beginning of each day using three phantoms of known density.

The subject lay on the couch with the hip and knees flexed to 45 degrees to minimize lumbar lordosis. The calibration phantom was already in position and lay under the subject with a standardised rubber pad in between. This ensured a fixed distance and a reduced air gap between the phantom and subject.

A lateral scout view of the lower thoracic and lumbar spine was obtained. The gantry of the scanner was then angled so that the slice plane was in the midpoint between the vertebral end plates. Slices were taken through the midpoints of the second, third and fourth lumbar vertebrae with the slice thickness being set at 5 mm. Although 10 mm cuts have been used by Cann and Genant (1980), we chose the smaller slice thickness in the belief that this would reduce the dose of radiation these young women received.

From the axial slices, the maximum area of purely trabecular bone in each vertebral body was defined using the circular computer-generated region of interest (ROI). This area always exceeded 200 mm^2 and care was taken to exclude the basi-vertebral vein and areas of cortical bone.

Positioning software was not used but on follow-up measurements attention was paid to match both the slice position on the lateral scout view and the region of interest (ROI) on the axial view of each vertebrae. The circular area measured was always the same as in the previous measurement but did vary between subjects.

Evaluation of the raw data and conversion into bone density equivalents was performed using regression analysis by Dr E McNally, Senior Registrar in Radiology.

ESTIMATE OF PRECISION OF THE TECHNIQUE

During the course of the study we had no reason to suspect problems with the precision of our technique, which was based on the method described by Cann and Genant (1980). The coefficient of variation given in chapter 3.4.3 is quoted from their studies. We have no "in-house" data on short or long term precision from the time of the study, but we have been able to calculate a measure of long term precision based on the available data. This has been done by studying the subgroup of athletes who remained eumenorrhoeic throughout the one year study period. It would seem reasonable to assume that the true physiological change in trabecular bone density of these athletes was insignificant and certainly smaller than in any other menstrual category. With the

proviso that this assumption is correct it has been possible to make an estimate of precision on the 13 athletes who fell into this category (tables A1, A2 and A3).

The coefficient of variation of the technique was determined by dividing the standard deviation of the difference between the measurements by the absolute mean of the measurements. The mean change in trabecular bone density in this group was -6.54 mg/cm^3 with a standard deviation of 17.37. The absolute mean bone density of the 2 measurements was 211.65 mg/cm^3. The coefficient of variation was therefore 8.2%.

The mean change and standard deviation yield a 95% reference range (Bland and Altman, 1986) of -40.6 mg/cm^3 to +27.5 mg/cm^3. Table A4 gives the number of athletes in each of the 4 menstrual categories (tables 10.2.1, 10.2.3, 10.2.5 and 10.2.7) who lie above and below this reference range. These are the athletes whose change in bone density is likely to be due to a true physiological effect.

POTENTIAL CAUSES OF BONE DENSITY CHANGES

The long term precision quoted in most studies is about 3% (Genant HK, 1990). Our relatively poor result may be due to several factors:-

1) True Physiological Changes which differed between subjects

2) Methodological Imprecision.

1) TRUE PHYSIOLOGICAL CHANGE

Over the course of one year there will be a small amount of true change in bone density. The training type and intensity of the athletes is likely to have changed during the year and this may have led to significant real changes in bone density. However this is unlikely to be large based on the information given in chapter 10.2 and table 10.2.14.

Furthermore, although these athletes remained eumenorrhoeic throughout the year, some of the cycles may have been anovulatory or had shortened luteal phases. This is thought to have a detrimental effect on bone density (Prior et al, 1990). The influence of these subtle changes in menstrual status on bone density were not assessed in our study but have been in a recent study by Prior et al (1990). They investigated bone density changes over a one year period in a group of 21 female marathon runners with regular menstrual cycles (although some were anovulatory and some had shortened luteal phases). Spinal bone density was measured by QCT from T12 to L3 at the beginning and end of a one year period. In many respects therefore, their study is comparable to our own although their athletes were slightly older (32.7 v 25.9 yrs), shorter (160 v 169 cm) and lighter (54.9 v 60.0 Kg) than the 13 athletes in our study.

The absolute mean bone density in their group was 153.3 mg/cm^3. The mean change in trabecular bone density was -3.0 mg/cm^3 with a standard deviation of 4.8. The coefficient of variation was therefore only 3.1%, compared to 8.2% in our study.

Based on this difference in the coefficient of variation, most of the change in trabecular bone density seen in our study is less likely to be due to subtle menstrual abnormalities or to changes in training habits and more likely to be due to methodological imprecision.

The levels of trabecular bone density seen in our study were high compared to other studies (table A5). The levels of bone density in the athletes in Prior's study (1990) were lower and this could be due partly to the age difference and possibly to differences in training intensity.

Marcus et al (1985) studied 17 female runners who were well matched with the athletes in our study in terms of age, height and weight (table A5) and aerobic fitness. Trabecular bone density amongst the amenorrhoeic athletes in both studies were similar. However amongst the eumenorrhoeics, our athletes had higher bone density levels and it is difficult to account for this.

Normal data, given by Block et al (1989), quotes a mean spinal trabecular bone density of only 166.7 (+/- 19.2) mg/cm^3 for non-athletic women in their twenties. Table A6 gives the mean

(standard deviation) for the amenorrhoeic, eumenorrhoeic and oral contraceptive-taking athletes and control group at the start of our study (table 5.5), and these are generally higher than the normal data quoted above. This disparity may be partly accounted for by the difference in physical activity.

Z-scores have been calculated for each of our athletes (i.e. [athlete's bone density - population mean]/ population standard deviation) based on Block's normal data. Table A6 shows how many athletes in each of the menstrual groups lie outside the 95% reference range (i.e. athletes with Z-scores above +1.96 or below -1.96). From this it can be seen that with the exception of 5 amenorrhoeic athletes, none of our subjects were below the reference range but 31 were above it.

While it is possible that the method we used might have over-estimated the true bone density, measurements in a sizeable group of normal non-athletic women (table A6) gave values which were similar to that found by others.

2) METHODOLOGICAL IMPRECISION

Methodological imprecision could be due to the following:-

a) Sampling errors

b) Errors related to Equipment.

a) Sampling errors

The imprecision may be due to errors in sampling. Prior et al (1990) evaluated bone density from 4 vertebrae (T12 to L3) taking a slice thickness of 8 mm. Measurements were taken from an elliptical region of interest from each of the 4 vertebrae as identified by an edge-detection programme.

We evaluated bone density from 3 vertebrae using 5 mm slices through each vertebrae which is regarded by some to be too small. Cann and Genant (1980) considered a slice thickness of 10 mm to be best as slices of greater than 13 mm may pick up vertebral end-plate whereas slices of only 5 mm could produce a significant error if there are inhomogeneities within the body of the vertebrae. Furthermore if on repeat measurement the slice is out by 1 mm, this will lead to a repositioning error of 20% if 5 mm slices are taken.

On the cross-sectional view of each vertebrae, we used a circle for the region of interest (ROI) which always exceeded 200 mm^2. Kalender et al (1987) suggested that an oval ROI was superior to a circular one and that an automated system for defining the ROI would improve the precision further.

Several studies have suggested that the area of the ROI and the volume of bone sampled should be maximized to reduce the repositioning error and improve precision (Banks LM & Stevenson

JC, 1986; Zamenhof RGA, 1987). The volumes used in our study always exceeded 1 cm^3 but usually were below 2.5 cm^3 whereas in other studies volumes tend to exceed 3.0 cm^3.

Without using positioning software, several studies have suggested the importance of using the same technician (two at the most) dedicated to the protocol (Banks LM & Stevenson JC, 1986; Adams JE & Adams PH, 1987). We used one of 3 senior radiographers who supervised more junior staff.

Errors in sampling therefore may have led to significant imprecision in our study. To assess the effect of sampling on the overall precision, we have looked at the change in each individual vertebra relative to the mean change of all 3 (ie L2, L3 and L4) - see table .3. This assumes that physiological effects and equipment effects, such as table height (see below), would influence each vertebrae to the same extent. Based on this assumption, large changes in each vertebrae relative to the mean change would imply errors in sampling (table A3).

The standard deviations of the changes in each individual vertebra are very similar to the standard deviation of the mean change (table A2) suggesting small sampling error. The standard deviation of the changes in each vertebra relative to the mean change (table A3) is 6.92 giving a variance of 47.89. This is small compared to the variance of the mean change in trabecular bone density (301.7). It is therefore unlikely that the imprecision is attributable to sampling errors alone.

b) Errors related to the equipment.

Several X-ray tube changes took place during the study and this may have affected the CT numbers. This should have been controlled for by the use of the phantom (Banks LM & Stevenson JC, 1986). However there has been some concern about the long-term stability of the aqueous solutions of K_2HPO_4 within the phantom due to the production of gas bubbles and precipitation of the dissolved materials. This has led to the development of a solid-based calibration phantom (Kalender WA & Suess C, 1987).

We used the liquid-based phantom. The regression line of the CT numbers of each of the 5 solutions against their known bone density equivalents remained very highly correlated throughout the study (R = 0.995 at the start and 0.996 at the end of the study). This demonstrates no significant deterioration of the phantom.

All athletes attended for assessment at random so that any changes in equipment characteristics with time would probably apply equally to each of the sub-groups studied.

Field uniformity is probably the most important requirement in a CT scanner used for QCT (Zamenhof RGA, 1987). Poor field uniformity can occur in scanners that employ reduced field-of-view scanning or use "bow-tie" x-ray filters. In such

circumstances change in table height is a significant source of variation (Morin et al, 1990; Faulkner KG et al, 1990). The GE 9000 scanner permits table height variation and also employs a "bow-tie" filter. By contrast, most of the Siemens scanners (including the Siemens DR2 used by Prior et al) have a fixed table height for all abdominal measurements. We did not control for table height although it is unlikely that this varied by more than about 2 cms. We did not record the table height with each measurement and therefore have no way of quantifying the degree of imprecision.

CONCLUSIONS

We have demonstrated substantial and highly variable changes in trabecular bone density over a one year period. Only a small proportion of these changes were likely to be due to a true physiological effect. The coefficient of variation was 8.2% which is higher than equivalent measurements in a similar study (3.1%). The lack of precision in the technique was predominantly due to 2 factors:-

1) Table height variability.

2) Sampling errors related to small sample volumes and to using several technicians to perform the procedure.

Despite the poor precision of QCT this does not invalidate the results from the cross-sectional study. There was no evidence that a systematic bias was introduced inadvertently into the study. We were still able to demonstrate significant differences between the menstrual (chapter 5) and sporting groups (chapter 6.1). We were also able to demonstrate a significant linear correlation with dietary calcium (chapter 8) and a non-linear relationship with VO_2max (chapter 7). The P-values would have been even more significant had the precision been better (Fleiss, 1986). The poor precision may also account for the lack of expected relationships displayed in the longitudinal studies (chapter 10.2).

Precision could have been improved by the following:-

1) Standardise the table height
2) Increasing sample volume to at least 3 cm^3
3) Using an oval ROI and positioning software (if available)
4) Using a maximum of 2 technicians to perform the measurements.
5) Determination of the short term precision of the technique prior to the project for the purpose of study design.

METHODOLOGY FOR DPA AND DEXA

FEMORAL MIDSHAFT BY DPA

The methodology for this technique was described in chapter 3.4.1.

The main limitation with DPA is that the radio-active source requires changing on a regular basis and therefore performance and calibration must checked frequently.

Performance is checked weekly against a standard which consists of an aluminium pipe (3 cm diameter) contained in 16 cm of water. This has a similar density to human femoral shaft.

The radio-active source is checked regularly by measuring the count rate from the two different energy channels. When the count rates fall below a predetermined level the source is changed. This occurs about once every 15 months.

When a new source is installed, the system requires calibration. This is done using a phantom which consists of 3 cylinders into which is placed solutions of known concentration of K_2HPO_4. These cylinders lie horizontally in 16 cm of water.

The source was changed once during the study, in between the first and second round of testing.

The position of the femoral midshaft is detected manually. Although this is imprecise, studies in our own laboratory (unpublished data) show that the femoral shaft bone density is unaffected by taking measurements up to 5 cms away from the midpoint. All the measurements were performed by one technician.

The coefficient of variation was assessed by doing repeated measurements on 8 subjects over a 5 day period. This short term precision was between 1 and 1.5%.

PROXIMAL FEMUR BY DEXA

The methodology for this technique was described in chapter 3.4.2.

Quality control measurements were made every day using a phantom containing 4 simulated vertebrae in a perspex box (17 x 15 x 18 cm). These vertebrae contain a homogeneous concentration of calcium throughout and lie down the central axis of the box. A measurement is taken from the phantom and expressed in grams of hydroxyapatite equivalent/cm^2. These readings have been taken almost daily for the last 2 years and have given a coefficient of variation of 0.4%.

Positioning is facilitated by placing the feet in a jig designed to internally rotate the hip. The subject lies down the centre line of the bed. At the first measurement the regions of interest are selected which are then matched up manually at subsequent visits. We did not use positioning software and the measurements were performed by one of two technicians dedicated to the task.

The coefficient of variation for the proximal femur values were estimated by taking several readings from 4 healthy volunteers over a 5 day period (ie short term precision). These were as follows:-

Neck	1.5%
Trochanteric region	1.5%
Ward's triangle	2.5%.

The reference ranges for these 3 sites of the proximal femur are given below. They are provided by Hologic and apply to normal women, aged 25 years. The standard deviation is given in brackets.

Neck	0.89 (0.10) gm/cm^2
Trochanteric region	0.72 (0.10) gm/cm^2
Ward's triangle	0.79 (0.11) gm/cm^2.

Tables 10.3.6 and 10.3.7 give the mean values for proximal femur measurements in the menstrual and sporting groups respectively. As can be seen they are very similar to the quoted reference range.

CONCLUSIONS

The precision of DEXA is outstanding while for DPA it is slightly less good (Genant et al, 1990). QCT is very operator-dependent requiring meticulous attention to detail otherwise reproducibility is impaired. As it can provide a precise measurement of trabecular bone density and good discrimination between controls and patients with postmenopausal osteoporosis, it has a valuable role in bone density research. The precision of the technique in relation to the expected changes should be considered at the design stage of a longitudinal investigation as this will determine the feasibility of the study and help to exclude the possibility of Type 2 errors.

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nb any references not listed here have already been included in chapter 12, entitled "References" (pages 247-279).

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TABLE A1

TRABECULAR BONE DENSITY OF EACH LUMBAR VERTEBRAE IN 1988 & 1989 IN EACH OF THE ATHLETES WHO REMAINED EUMENORRHOEIC.

REG NO	SPORT	L2 (mg/cm^3)	L3 (mg/cm^3)	L4 (mg/cm^3)	MEAN VALUE (mg/cm^3)
			1988		
009	Ro	244	232	249	242
017	Ro	213	204	214	210
020	Ro	176	197	215	196
021	Ro	183	191	221	198
032	Ro	222	244	249	238
033	Ro	194	201	201	199
040	D	219	204	218	214
051	Ru	203	210	255	223
055	D	213	200	206	206
056	D	179	176	186	180
058	Ro	213	219	241	224
059	Ru	262	270	266	266
070	Ru	189	193	210	197
			1989		
009		205	202	211	206
017		232	229	229	230
020		179	203	225	202
021		160	171	205	179
032		232	244	260	245
033		195	199	184	193
040		235	213	214	221
051		193	194	209	199
055		217	201	203	207
056		171	155	160	162
058		212	215	226	218
059		240	238	234	237
070		210	202	220	211

TABLE A2

CHANGE IN TRABECULAR BONE DENSITY OVER ONE YEAR IN THE 13 EUMENORRHOEIC ATHLETES

REG NO	CHANGE IN L2 (mg/cm^3)	CHANGE IN L3 (mg/cm^3)	CHANGE IN L4 (mg/cm^3)	MEAN CHANGE (mg/cm^3)
009	-39	-30	-38	-36
017	19	25	15	20
020	3	6	10	6
021	-23	-20	-16	-20
032	10	0	11	7
033	1	-2	-17	-6
040	16	9	-4	7
051	-10	-16	-46	-24
055	4	1	-3	1
056	-8	-21	-26	-18
058	-1	-4	-15	-7
059	-22	-32	-32	-29
070	21	9	10	13
MEAN	-2.23	-5.77	-11.62	-6.54
St. Dev.	17.84	16.91	20.10	17.37

TABLE A3

CHANGE IN TRABECULAR BONE DENSITY IN EACH VERTEBRAE RELATIVE TO THE MEAN CHANGE.

REG NO	L2 (mg/cm^3)	L3 (mg/cm^3)	L4 (mg/cm^3)
009	-3	6	-2
017	-1	5	-5
020	-3	0	4
021	-3	0	4
032	3	-7	4
033	7	4	-11
040	9	2	-11
051	14	8	-22
055	3	0	-4
056	10	-3	-8
058	6	3	-8
059	7	-3	-3
070	8	-4	-3
MEAN	4.31	.82	-5.08

TABLE A4

NUMBER OF ATHLETES IN EACH OF THE 4 MENSTRUAL CATEGORIES WHO LIE ABOVE AND BELOW THE 95% REFERENCE RANGE FOR CHANGE IN TRABECULAR BONE DENSITY

(Definitiions of menstrual categories given on page 189)

	AA	NN	AON	NO	TOTAL
Number	15	32	9	5	61
Number below 95% Ref. Range	0	4	0	1	5
Number above 95% Ref. Range	1	3	1	0	5
Number outside 95% Ref. Range	1	7	1	1	10

TABLE A5

COMPARISON OF MEAN (+/- SD) TRABECULAR BONE DENSITY LEVELS IN OUR STUDY AND IN STUDIES BY MARCUS ET AL (1985) AND PRIOR ET AL (1990)

	OUR STUDY	MARCUS ET AL (1985)	PRIOR ET AL (1990)
SPORT	Runners & Dancers	Runners	Runners
EUMENORRHOICS			
Number	12	6	21
Age (yrs)	25.5 (3.4)	23.8 (1.7)	32.7 (5.9)
Ht (cm)	165 (4)	164 (1)	160 (6)
Wt (Kg)	54.3 (5.5)	53.8 (1.6)	54.9 (6.4)
TBD (mg/cm^3)	206 (29)	182 (5)	155 (20)
AMENORRHOEICS			
Number	15	11	*
Age (yrs)	24.3 (3.4)	20.0 (0.4)	*
Ht (cm)	162 (5)	164 (2)	*
Wt (Kg)	48.2 (3.7)	49.7 (1.5)	*
TBD (mg/cm^3)	156 (43)	151 (8)	*

TABLE A6

TRABECULAR BONE DENSITY OF THE MENSTRUAL AND CONTROL GROUPS IN OUR STUDY GIVING THE NUMBER OF SUBJECTS LYING OUTSIDE THE 95% REFERENCE RANGE (BLOCK ET AL, 1989)

	AMENORR-HOEICS	EUMENORR-HOEICS	ORAL CONTRA. TAKERS	CONTROL GROUP	TOTAL
NUMBER	25	27	15	30	80
MEAN (sd) TBD (mg/cm^3)	168 (38.2)	211 (30.3)	215 (36.5)	187 (25.5)	
Number of Subjects:					
$Z < -1.96$	5	0	0	0	5
$Z > +1.96$	4	12	11	4	31
Outside 95% Ref. Range	9	12	11	4	36