1994

The response of trabecular bone to physical activity in young sedentary males

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THE RESPONSE OF TRABECULAR BONE TO PHYSICAL ACTIVITY IN YOUNG SEDENTARY MALES

A thesis submitted in fulfilment of the

requirements for the award of the degree

MASTER OF SCIENCE (Honours)

from

UNIVERSITY OF WOLLONGONG

by

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DEPARTMENT OF BIOMEDICAL SCIENCE

MARCH, 1994.

ABSTRACT

Bone is a dynamic tissue that is continuously undergoing cycles of resorption and formation throughout life. Factors known to effect this remodelling process include an individual's nutritional and hormonal status and physical activity and achievable bone density is dependent on the interaction between an individuals chosen lifestyle and genetic make-up. There is considerable evidence that physical activity may have a positive effect on bone mineral density through the effects of mechanical loading and local and systemic physiological mechanisms. Lack of a change in bone mineral density following exercise has also been found, and this discrepancy in the literature may reflect differences in the nature, intensity and duration of the exercise programs that have been used. The complexity of the interaction between lifestyle and genetic factors, age and site specific responses also makes interpretation of the literature difficult. It has been suggested that attainment of a high peak bone mass earlier in life may compensate for the normal loss of bone which occurs and accelerates with aging. Moderate intensity activity has been shown to have a positive effect in the development of skeletal mass in children. However, there is a paucity of prospective information on the effects of physical activity in early-adulthood. The aim of this study was to investigate whether a protocol of running training designed to increase cardiovascular fitness would effect changes in bone mineral density (BMD), bone mineral content (BMC) and bone metabolism of young sedentary males. This study was also designed to assess possible associations between cardiovascular fitness and anthropometric variables and BMD and BMC.

Twenty-six sedentary males with an average age of 22.2 years volunteered to participate in this study. Sixteen of those chosen to participate undertook a 16 week program of progressive running training and as the exercise program progressed the running distance became longer and the intensity of the runs was increased. Ten males acted as the control group and did not participate in any organised program of physical activity. Physical activity diaries were kept by all experimental and control subjects in order to assess daily training histories of both formal and informal activity. A maximal exercise treadmill test

was conducted to assess the cardiovascular fitness of all subjects and establish the training intensities for the exercising subjects. Tests were conducted before commencement of the program, at 4 weekly intervals and immediately following the intervention period. Cumulative heart rates and time to exhaustion from the initial and final maximal treadmill test were used as indicators of a change in cardiovascular fitness over the 16 weeks. Bone mineral density and content was assessed at three sites on the proximal femur and in lumbar vertebrae 2 to 4 using dual energy x-ray absorptiometry (Norland XR-26). The biochemical markers of bone formation and resorption, namely serum osteocalcin and urinary hydroxyproline were used to evaluate the effects of the exercise program on bone metabolism.

No differences were found between the exercise and control group for bone mineral density and bone mineral content at the majority of skeletal sites measured. The exception to this finding was a significant decrease in BMD and BMC in the 4th lumbar vertebrae between exercise and control groups following the training program. Urinary hydroxyproline and serum osteocalcin, markers of resorption and formation respectively did not change following the training program and showed no relationship with BMD or BMC. The exercising subjects experienced a significant increase in cardiovascular fitness following the 16 week training program, however, no association was found between cardiovascular fitness and BMD or BMC. The anthropometric measures of weight and height were both found to correlate with BMD and content for sites on both the proximal femur and lumbar spine.

The results of this investigation have shown that a short-term exercise program of moderate intensity did not stimulate the acquisition of BMD or BMC of young males. There was no differences between the exercise and non-exercise groups, in spite of differences in the intensity and duration of their activity. This suggests that for normal maintenance of BMD a broad range of physical activity exists, before the threshold for acquisition of bone is reached. As such exercise program of longer duration with a

similar and/or greater intensity may be required to reach this threshold and effect increases in bone mineralisation of the proximal femur and lumbar spine at this age.

ACKNOWLEDGMENTS

I would like take this opportunity to thank my supervisor Professor Tony Parker for his guidance, support and continual encouragement in writing this thesis, without his support and friendship this thesis would not have become a reality. Many thanks to my associate supervisor Dr Nigel Taylor for his help and guidance in various aspects of this work. I would also like to acknowledge Dr Stephen Boutcher for his advice on the statistical procedures. Thank you to Dr Lyn Lee for her help in collecting blood from the subjects and her ideas for running a successful intervention program.

I am indebted to the assistance I have received from Illawarra Nuclear Imaging in Wollongong, for allowing me use of the Norland bone densitometer, especially from Dr Richard Mclean and Kerrie Newman for her expert technical assistance. I am especially grateful to the staff of Sugerman's Pathology in Sydney and I would like to acknowledge and thank Rick Symons, Frank Facchian, Rod Frazer and Mari-anne Hirn for the time they made for me in the laboratory and their expertise in guiding and assisting me with the biochemical assays used in this thesis.

To the 22 guys who made this thesis a reality, and the 12 young men with the determination to finish the running program, thank you so much.

Thankyou to my esteemed postgraduate colleagues Mark Osbourne, Glenn Doney, Graeme Maw, Euan Tarleton, Bruce Power and Jennifer Broughton for the help, humour and coffee breaks you provided me with during our time together in Wollongong.

Finally, I an extremely grateful to my family, my parents Bernice and Matthew and my sisters Lara and Romy in providing me with all the love, support and guidance a son and brother could ever need.

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CHAPTER 1

INTRODUCTION

Bone is a dynamic tissue that is constantly adapting its composition and architectural structure to mechanical loading. This process of adaptation begins early in fetal development (Carter et al. 1987) by the actions of muscles and continues throughout life in response to the level of loading inherent in the individuals' activity profile. Genetic factors are estimated to contribute approximately 80 per cent of adult bone mass and density (Pocock et al. 1987), and contributions are also made by environmental and lifestyle influences, which may have a positive or negative effect on bone. The density of bone is considered an important indicator of skeletal health reflecting both positive and negative adaptation. Several important environmental factors that may effect bone density include dietary calcium, cigarette smoking and alcohol use, and physical activity may be the single most important lifestyle influence for an individual's continued bone health (Drinkwater, 1993).

There is considerable information which indicates that a bone's density is related to the type, intensity and duration of the physical activity or mechanical loading to which it is subjected. An early study by Nilsson and Westlin (1971) examined the bone density of different athletic groups. The degree of mineralisation was associated with the apparent level of loading induced by the sport, with the highest bone density found in weightlifters followed by throwers, runners, soccer players and swimmers. Although changes in bone density reflected the apparent intensity of the activity, the athlete's chronic involvement in weight-bearing activity over time may have been largely responsible. This is clearly seen in tennis players with over 25 years of playing experience, whereby greater radial hypertrophy and bone mineral content was found in their dominant compared to their non-dominant arm (Jones et al. 1977; Huddleston et al. 1980). Activities in which the influence of gravity has been reduced such as swimming have also elicited an increase in bone density (Orwoll et al. 1989). The positive influence of swimming on bone density

demonstrates the importance of direct muscle force acting on the bone surface and its stimulatory effect on bone remodelling.

Although limited in number longitudinal training studies have provided information of the efficacy of different exercise intensities and the response of bone in a number of age categories. Several longitudinal studies have investigated the effects of moderate intensity running activity on middle-aged sedentary men and women (William et al. 1984; Dalsky et al. 1988; Nelson et al. 1991). The results of these studies indicate that this type of activity was associated with increased bone density following 9 months of training. When the duration was decreased to 3 months there was no change in the bone density of males aged 25-52 years (Dalen and Olsson, 1974). When the intensity of the exercise was increased a change in bone density was seen following a short 14 week intervention in young males aged 18-21 years (Leichter et al. 1989).

A majority of intervention studies have attempted to assess the effectivness of increased physical activity on the prevention or reduction of bone loss which occurs as a normal process of aging (Simkin et al. 1991; Talmage et al. 1986). They have shown physical activity to be effective at reducing the rate of bone loss with age and in some cases has increased bone density. These investigations relied on relatively long duration exercise programs of 12 months or longer to exert such an effect.

In contrast to the positive adaptation associated with exercise, reduced loading environments, for example bed rest or weightlessness, have a degenerative effect on bone mineral density. Substantial decreases in bone mass are evident when normal mechanical loading is withdrawn, as for example in the os calcis of astronauts following 14 days of spaceflight (Mack et al. 1967). Significant bone loss of approximately 45 per cent of the os calcis has been reported in men following up to 36 weeks of bed rest (Donaldson et al. 1970). Fortunately reversal of loading patterns can restore bone mass to its original levels, which in the latter study occurred over a period of 36 weeks.

The development of a high bone density early in life may provide a safety factor and compensate for the normal loss of bone associated with aging, which is accelerated around post menopause in women. During the adolescent growth period there is a large variation in growth rates and once skeletal maturity is reached the maximum amount of bone mineral in an individual bone at this time is termed peak bone mass. The age at which peak bone mass and density is attained is uncertain, although evidence generally supports the third decade of life (Geusens et al. 1986; Gotfredsen et al. 1989; Rico et al. 1992). The importance of an active childhood on bone health has been shown through retrospective activity studies of adults (Kriska et al. 1988; Slemenda et al. 1991). Those adults that participated in extensive physical activity as children have a significantly greater bone density than those with an inactive childhood. There is little evidence of the response of bone to exercise in young adulthood and exercise studies that have been conducted on individuals in the 3rd decade of life have either utilised exercise intensities that have been responsible for an increase in both bone density and stress related injuries (Margulies et al. 1986; Leichter et al. 1989) or subject samples have included those over the 3rd decade (Dalen and Olsson, 1974). Few researchers have chosen to address the problem of age-related bone loss from a preventive perspective in this age group, and there is a particular lack of information on the effect of exercise of moderate intensity of shorter duration.

There is evidence from studies of mature adults and the elderly that physical activity has a positive influence on bone density. However, evidence from similar studies involving young adults, particularly post adolescents when peak bone mass is assumed to occur is limited. There is a need to extend our knowledge of the effect of exercise during this period of life. Therefore the purpose of this investigation was to evaluate the effects of a 16 week running training program on bone composition and bone metabolism in young sedentary males aged 20-27 years. The 3rd decade of life is unique in that growth is completed (Bass, 1971), however there may still be a potential lag in mineralisation. Investigation of the skeletal response to moderate intensity exercise of relatively short

duration during this time may show the appropriateness of the prescribed exercise at this age level. The specific aims were to investigate whether;

(1) bone mineral density and bone mineral content at sites on the lumbar vertebrae and proximal femur would change following the exercise program.

(2) biochemical estimates of bone metabolism would change following the exercise program.

(3) an association exists between bone mineral density and bone mineral content and cardiovascular fitness and anthropometric variables.

CHAPTER 2

REVIEW OF RELATED LITERATURE

This review is introduced by details of the basic biology of bone including the organs internal and external structure and the process of bone mineralisation. The process of bone mineralisation is followed from the early years of bone growth up to maturity followed by the effects aging has on bone tissue. An understanding of the developmental phases of bone mineral apposition allows correct interpretation of the changes that may occur in a longitudinal investigation involving an exercise protocol. This includes comprehension of the principles governing the mechanical loading of bone examined through the early animal studies and the more recent principles on bone's response to loading stimuli. The effect of exercise as a stimulus to bone mineralisation and the mechanisms used to measure bone mineral content and density and to monitor bone turnover are also included in this review.

The human skeleton consists of cortical and trabecular bone. The former comprises the compact layer that forms the diaphyses of long bones, whereas trabecular bone forms the interior mesh of the bone and is found in the epiphyses and medullary cavity of long bones. Water accounts for about 20 per cent of the wet weight of mature cortical bone, bone salts about 45 per cent and the organic matrix the remaining 35 per cent. The principal chemical constituents of bone mineral are calcium phosphate and carbonate, with lesser quantities of sodium, magnesium and fluoride (Carter and Spengler, 1978). They are present as a mixture of hydroxyapatite crystals and amorphous calcium phosphate. The organic matrix of adult bone tissue is comprised of 90 per cent type I and III collagen and 10 per cent ground substance. The non-collagenous component is small in amount and apart from lipids and peptides it is largely composed of carbohydrate protein complexes (glycoproteins) and proteoglycans (glycosaminoglycans) (Vaughan, 1981).

The surface of a bone is continually being remodelled throughout life with the process involving resorption of bone from one site and deposition of bone in another location. This remodelling leads to ultrastructural changes including the complete loss of trabecular struts and ties, enlargement of the medullary cavity, increased size of the vascular canals and trabeculation of cortical bone (Compston et al. 1989; Martin and Burr, 1989). The process is mediated by the activity of three specific bone cells; osteoblasts, osteocytes and osteoclasts (Rasmussen, 1974). Osteoblasts are responsible for the synthesis of the extracellular bone matrix components and for priming the matrix for mineralisation. Osteocytes are derived from osteoblasts and lie within the mineralised bone. They are usually arranged in concentric layers with osteons (Haversian systems) consisting of layers of tissue around a vascular canal. This arrangement provides a source of nutrition for the cells and for the mobilisation of minerals. Osteoclasts lie on the surface of the bone and are associated with the resorption of calcified bone or cartilage beneath the periosteal surface (Russell et al. 1983).

Changes in Bone Composition with Age

The mineralisation of bone tissue commences in the fetus at approximately 2 months of age (Carter et al. 1987) and continues throughout childhood until the time of epiphyseal plate closure. Glastre et al. (1990) examined early mineralisation patterns of L1 to L4 in boys and girls between the ages of 1-15 years using dual energy x-ray absorptiometry. The BMD of the lumbar spine increased significantly with age in the children, with the steeper increase at the time of puberty. At the age of 12 years BMD was higher in girls, and at 15 years the normal mean BMD was 0.89 $g.cm^{-2}$, about twice that at 2 years of age. A group of similar aged children was studied by Miller et al. (1991), and the analysis was extended to include the wrist and proximal femur. There were no differences between boys and girls in mineralisation at the radial site, but girls had a greater bone density in the spine and less in the Ward's triangle region on the proximal femur. This study also tested the relationship between anthropometric variables and BMD and found height to be the single best predictor of BMD for both male and females.

Circumferences of the upper arm, abdomen, hip, and calf were also found to be significant predictors of BMD at the radius and femoral sites. The results of this study demonstrate that changes in bone mineralisation are associated with growth in children. It was argued however, that the taller children matured earlier, which may account for height being the single most important predictive factor for BMD.

In an investigation of the critical phases of bone growth Bonjour et al. (1991) assessed the change in mineralisation of the skeleton in 207 caucasian boys and girls, aged 9-18 years. Bone mineral density and content was determined in the lumbar spine, femoral neck and mid-femoral shaft, using dual energy x-ray absorptiometry. There was no difference in bone mineral density of boys and girls between the ages of 9-10 years. Girls aged 12-15 years had a higher mean BMD than males of the same age, at each skeletal site. This earlier development in girls was particularly pronounced at L2-L4 whereas at 17-18 years of age there was no significant difference between sexes at this site. There was a trend for greater BMD in males than in females at the femoral neck and mid-femoral shaft at this age. Bonjour and co-workers added qualified support to the findings reported earlier (Miller et al. 1991) in showing a positive relationship between height and BMD/BMC. They also found this relationship was no longer evident at a height of approximately 155 cm in females and 160 cm in males, which corresponded to an average age of 13 years and 13.5 years in females and males respectively.

The same authors then contrasted the BMD of adults aged 20-35 years with the values attained in the 9-18 year age group. Bone mineral density and content of the 17-18 year male group was not significantly different from the 20-35 year group, whereas females aged 14-15 years had already achieved the BMD of their adult counterparts at the lumbar spine and femoral neck. This data is supported by Gilsanz et al. (1988) who found no significant differences between a group of 14-19 year old adolescent females compared to a group of young adults, aged 25-35 years. The results of these investigations indicate early bone mineral deposition in the adolescent years for both sexes, however,

there was also an apparent site specificity in mineralisation of the developing adolescent bone.

Individual bones mature at different rates and the maximum amount of mineralisation reached in the skeleton of an individual bone around skeletal maturity is referred to as peak bone mass. A discrepancy exists in the literature concerning the age for the attainment of peak bone mass which reflects either gender differences or site specificity. Therefore when interpreting results one must take into account the age of the subject and the site of the density or content measure. A study of male and female subjects aged between 3 and 30 years (Gordon et al. 1991) found that in males, growth during puberty contributes little to peak bone mass in the lumbar spine as bone mineralisation at this site was still increasing at age 30 years. However in females, approximately half of peak bone mass at this site was accumulated at the time of puberty and was complete following longitudinal growth. Their results showed that in females, changes in mineralisation during puberty accounted for 39 per cent for BMD and 55 per cent for BMC. In contrast, for males the influence of puberty was smaller for both BMD (11 per cent) and BMC (21 per cent). The age of attainment of peak bone mass of women in the latter study is supported by Gilsanz et al. (1988) who concluded that peak bone mass in the lumbar spine is achieved by the age of 20 years in females, around the time of cessation of longitudinal growth. Gotfredsen et al. (1987) reported a decrease in total body peak bone mass in males after the age of 20 years, which is a direct contrast to the results of Gordon and co-workers. Geusens et al. (1986) reported that peak bone mass measured at the radius and lumbar spine is reached at the age of 25 years in men. In women, peak bone mass of the spine is reached at age 25 years, but peak bone mass at the radius occurs 10 years later, indicating a differential development pattern between sites and sex.

The attainment of high bone density during earlier growth periods may provide a reserve of bone mineral which acts as a safety margin to compensate for bone loss which occurs

later in life (Bailey and McCulloch, 1992). Although it has been suggested that exercise may be a critical factor in attaining a high peak bone mass during growth, no evidence has been found to support this, or whether other factors such as heredity, race, gender (Boyd Eaton and Nelson, 1991) and diet have a greater influence. An adequate dietary intake of calcium is believed essential to satisfy extra skeletal needs during growth and for the attainment of a high peak bone mass (Matkovic, 1992). However it is not known how much of an effect environmental or lifestyle factors have on an individuals' genetic profile in order to develop their peak bone mass. A study of identical adult twins with differing lifestyles has shown that the genotype may influence up to 80 per cent of the variance in bone mineral density of the lumbar spine and femoral neck (Pocock et al. 1987). If this figure is accurate then exercise may play an important role in the individual reaching their maximal potential. Additionally, gender differences in hormonal profiles influence bone size and density. Androgens increase and maintain bone mineral density in males during growth and adulthood, following which there becomes an age-related decline. Estrogen levels in females during the growing years supports the apposition of bone mineral until menopause, where a decrease in estrogen levels leads to a reduction in bone mineral density (Geusens et al. 1991). Therefore an exercise program operating at the correct intensity and duration may provide a valuable opportunity by which to increase bone mineralisation in young adults, and promote gains in peak bone mass as a protective mechanism, and compensate for the decline in BMD with age.

It has been well established that there is a net loss of cortical and trabecular bone with increasing age. The walls of trabecular bone become thinner and may even be reabsorbed leading to a decrease in cortical thickness and strength (Rudd et al. 1989). Examination of trabecular and cortical bone density in the radius was undertaken by Ruegsegger et al. (1991) using computed tomography in healthy females ages 20-70 years. Trabecular density in young females with a mean age of 24 years was significantly greater compared to the older women with an average age of 67 years. Cortical bone density in the young females varied little from that in the older population,

however it was significantly higher than trabecular bone density. The authors concluded that loss of trabecular bone density in aging females was caused by alterations in the size and number of trabeculae within the sample volume, compared with the unchanged microstructure of cortical bone. Similar age-related decline in trabecular density was reported by Riggs et al. (1982) who measured bone mineral density of the proximal femur and lumbar spine in women and men, aged 20-92 years. Age regression analysis on BMD in women showed the predicted mean BMD in the proximal femur at age 90 years was reduced by 58 per cent compared to women aged 20 years and 42 per cent for the lumbar spine. In women, the proximal femur and lumbar spine had a lower BMD than that observed in men. It was suggested that this difference may explain why the female/male ratio for hip fractures is 2:1, whereas for vertebral fractures it is about 8:1(Sinaki, 1989). The result of these studies suggest there is a large individual variation in the age at which significant bone loss becomes evident in both males and females, which may reflect the higher or lower BMD levels accumulated prior to skeletal maturity.

The disparity between trabecular and cortical bone loss was examined in healthy men aged from 30 to 92 years (Meier et al. 1984). Radial and vertebral bone mineral content was measured, as they contain areas of high cortical and trabecular bone respectively. Bone mineral content declined significantly at all sites as a function of age. At the proximal radius there was a decline of 2 per cent per decade, and the distal radius declined by 3.4 per cent per decade, as a result of its higher trabecular bone content. Vertebral bone mineral content declined more rapidly with age, at 12.2 per cent per decade. In contrast with earlier results, this investigation showed a marked decline in bone mineral content in healthy men, which is inconsistent with previous conceptions of bone loss in men (Riggs et al. 1981, 1982). A disparity between the sexes concerning bone loss reported by Parfitt (1988) has shown males experience an average cortical bone loss of approximately 0.3 per cent of acquired bone mass per year, with trabecular loss being slightly faster. Females lost approximately 1 per cent of acquired bone mass

per year for both cortical and trabecular bone, with acceleration for around five years following menopause due to the disruption of estrogen.

Mechanical Loading of Bone

Throughout life, bone is subjected to mechanical loading of varying magnitude and frequency. Loading provides a stimulus for bone growth, bone modelling and remodelling and there is a direct relationship between the structural and mechanical properties of a bone and its function. Longitudinal bone growth adds new primary spongiosa and new length to the cortices. Bone modelling sculptures the shape and size of the cortices during growth, and remodelling controls bone turnover and replacement through osteoblastic and osteoclastic activity (Frost, 1987). A solid object is deformed when loads are applied to its surface. The same occurs in bone tissue as it becomes responsive to the mechanical demands imposed upon its tissue surface which in turn create internal forces. The deformations created in bone tissue are referred to as strains and the internal force intensities are referred to as stresses (Hayes, 1991).

Evans (1953) first recorded bone strain in an animal limb during gait. A single strain gauge was applied to a canine tibia, which was left open to limit exposure to moisture. This study and other earlier research (Lanyon, 1971, 1972) were limited by the single strain gauges that could only provide strain measurements in the direction the gauge was aligned. To resolve this limitation Lanyon (1973) developed a strain gauge rosette composed of three single gauges which allowed strains to be recorded at different angles. The first trial was on the calcanei of sheep and was used to calculate the magnitude and direction of the principal strains during walking and trotting on a treadmill belt. In a later study (Lanyon et al. 1975) the technique was used to examine the deformation of the human tibia during running and demonstrated that the tibia was subjected to a number of discrete deformation cycles. During each cycle the bone was deformed in the direction of the stress, with the largest deformation occurring while the subject was running, possibly due to greater ground reaction forces. The strain values recorded in this

experiment were comparable to those previously recorded from the long bones of sheep. However, the experiment was limited by the placement of the strain gauges, as the strain histories were not measured at the location of highest stress, the antero-lateral surface. Strains were only measured in a small area of the tibia and therefore would not have provided a representation of deformation over the entire bone length.

The relationship between stress and the remodelling response of bone was investigated in the ulnae of adult dogs (Chamay and Tschantz, 1972). In one group of dogs a small section of the radius was resected causing the animals to walk on their weakened forepaws, with all the load being taken through the ulnae. This resulted in either a fatigue fracture or bone hypertrophy, depending on the activity of the dogs. Those with fatigue fractures were removed from the study, and the remainder were studied from a further 1 day to 9 months. Several hours after activity was resumed, oblique lesions were found on the concave or compressive side of the ulnae, and at 48 hours new periosteal bone was visible. After 9 weeks the cortical thickness was increased by 60-100 per cent. The remodelling process progressed over the 9 month period and resulted in a considerably enlarged diaphysis.

This relationship was again examined in young pigs following ulna ostectomy (Goodship et al. 1979). After three months there was a rapid and substantial remodelling response in the radius as its cross-sectional area became equal to the combined areas of the radius and ulnae in the opposite limb. The authors suggested that the marked adaptive response in the bone was due to the frequency and number of cycles, the amount of strain, its rate and the duration of the strain with each cycle. The results of these studies demonstrate the sensitivity of bone remodelling to strain distribution and magnitude.

To confirm the association between dynamic strains and remodelling activity Rubin and Lanyon (1984) developed an experimental avian model designed to isolate and control

the application of strains of varying intensity, frequency and duration. Isolation of the rooster or turkey (Rubin and Lanyon, 1984; Lanyon and Rubin, 1984) ulna from normal muscular loading was achieved by osteotomy of the ulna at the epiphyseal-metaphyseal junction. The ends of the bone shaft were covered with stainless steel caps which were pierced by stainless steel pins. The pins were inserted parallel to one another and emerged on the dorsal and ventral surface of the wing. Application of controlled forces through these pins allowed for daily periods of intermittent loading while the contralateral bone served as a control. Bones that were loaded for 4 cycles per day remained essentially unchanged over the 6 week experimental period. When subjected to 36 cycles per day the ulna showed a rapid increase in bone mineral content following 2 and 3 weeks of loading, with values peaking around 28 days and stabilising by 6 weeks. The increase in bone mineral content in bones subjected to 360 cycles followed the same pattern as those subjected to 36 cycles. The results illustrate the sensitivity of the remodelling response of bone to a small number of strain cycles. The same model was used to assess the effects of disuse, disuse with a superimposed continuous compressive load of 525 N and disuse interrupted by a short period of 100 cycles of intermittent compressive load of 528 N. After an 8 week period the non-loaded and statically loaded bones increased their cortical porosity and showed an overall decrease in cross-sectional area. In contrast, intermittently loaded bones showed a 24 per cent increase in cross-sectional area, and deposition of new bone in the periosteum. The results showed that bone responds positively to intermittent loading patterns and this illustrates that the most substantial benefit upon bone mineralisation will occur from specific activities such as those of walking, jogging and running. Static loads had a detrimental effect on the structural integrity of the bone examined.

To explain the mechanism of the bone remodelling response to mechanical loading Frost (1983) proposed that strain rather than stress imposed on a bone initiates a feedback mechanism which leads to either bone apposition or resorption. He suggested the minimum effective signal that invokes the feedback mechanism would be the minimum

effective strain (MES) required for bone formation. The feedback mechanism is activated by a mechanical stimulus which is transduced into a biochemical signal. This in turn controls the cellular response to bone remodelling (Frost, 1983). Adaptations to the architecture of bone would occur for those strains larger than the MES. For example, weight bearing activity may generate the required loads or stress above the MES that will encourage increases in bone density and activate the processes to cause bone remodelling (Whalen et al. 1988). In a later article Frost (1993) suggests the control of threshold ranges may be due to genetically adopted "set points".

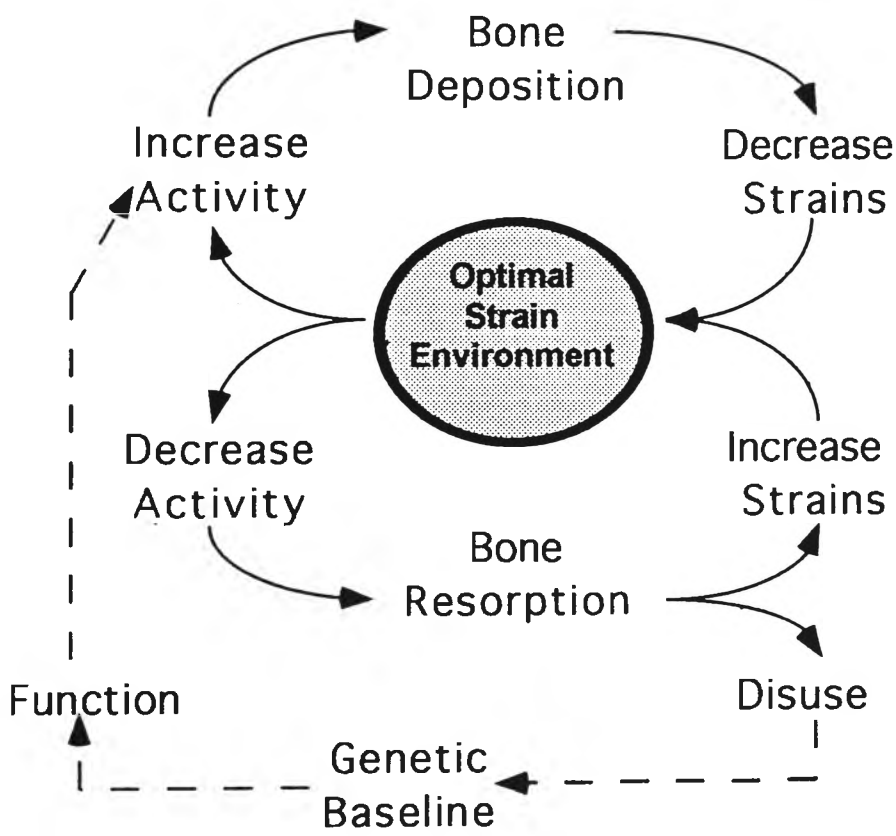


Figure 2.1. Diagrammatic representation of the negative feedback system responsible for the control of the remodelling cycle in bone tissue (Adapted from Rubin, 1984).

Bone Mineral Density and Exercise

There is considerable evidence to support the hypothesis that physical activity provides a stimulus above the MES which can be responsible for the changes which occur in bone composition. An early study by Nilsson and Westlin (1971) examined the bone density of different athletic groups. The groups included top ranked athletes which were compared with control groups consisting of regularly active and inactive healthy men. Their results showed that individuals who trained regularly had a higher density of the distal femur than non-athletes. The degree of mineralisation was associated with the apparent level of loading induced by the sport, with the highest bone density found in weightlifters followed by throwers, runners, soccer players and swimmers. Bone density of the swimmers did not differ from the inactive control group. In the control group, individuals who regularly exercised had a significantly greater density than those who were inactive. Although changes in bone density reflected the apparent intensity of the activity, moderate activity was still a positive influence on bone density.

Exercise studies of young males have often been overlooked in preference to the more widely studied field of bone related disorders such as osteoporosis which predominantly effect women. Virvidakis et al. (1990) has provided evidence of increased mineralisation in the non-dominant forearm of competitive weight-lifters aged from 15-20 years. Forearm BMC of the athletes was more than two standard deviations above the mean of the controls. The most important single variable in explaining BMC variations was body weight, which could be expected to strongly relate to muscle mass in the weight-lifters. It was concluded that the vigorous training undertaken by young weight-lifters is sufficient to increase BMC, possibly due to the added tension generated by the active muscles on the surface of the bone, and as a result of an increased muscle mass.

The effect of different loading activities was studied in 41 young men, 28 of whom engaged in regular and vigorous exercise programs (Block et al. 1986). Trabecular bone mineral density and bone mineral content of the L1 vertebrae in the exercise group was

greater by 14 per cent and 11 per cent respectively, when compared to that of the control group. Analysis of activity questionnaires completed by each subject found those that engaged in a program involving both weight-bearing and aerobic forms of exercise had the greatest bone density followed by those engaged purely in weight-bearing exercise. They were followed by men who participated in aerobic exercise only, with the control group found to have the lowest bone density of all the groups.

Information concerning the adaptability of bone to very intense training programs has been derived from studies of military recruits (Margulies et al. 1986). The recruits were 18-21 year old males and were required to participate in walking and jogging with and without weights, and callisthenics for at least 8 hours a day, 6 days a week, for a total of 14 weeks. During the training period the average BMC of the left leg, measured in the distal third of the tibia increased 11.1 per cent and that of the right by 5.2 per cent. The authors noted that the high level of loading either resulted in a rapid increase in BMC or a stress fracture. The results of Block et al. (1989) and Margulies et al. (1986) suggest that a high intensity exercise (loading) is required in order to enhance peak bone mass acquired following longitudinal growth. However, the individual differences in potential for adaptation following exercise and the previous loading history of each individual are important considerations. Skeletons that have not experienced high functional loads may have an increased susceptibility to injury, more specifically stress fracture. An example of this process was shown in adolescent gymnasts where the distal wrist growth plate was unable to withstand rotational and compressive forces which resulted in the premature closure of the distal radial epiphysis (Carter and Aldridge, 1988; Albenese et al. 1989).

Several studies have failed to find a relationship between exercise and bone density which may be the result of an underestimation of the exercise type, intensity and duration. For example, Cavanaugh et al. (1988) studied a group of postmenopausal women aged 49-64 years who participated in an endurance walking program for 15-40

min/day, 3 days/week, for 12 months. The rate of bone loss over the year was no different between control and exercise group. It was suggested that the intensity and frequency of this program may have been insufficient to stimulate the deposition of bone or maintain existing bone levels.

A 3 month program of exercise was devised by Dalen and Olsson (1974) in young sedentary male office workers. Two exercise groups were formed, those subjects that walked 3 kilometres 5 times a week, and those that were required to run 5 kilometres, 3 times a week. Bone mineral content was unchanged at the distal end and shaft of the radius and ulna, head of the humerus, third lumbar vertebrae, shaft and neck of the femur and the calcaneus following both programs. However the exercise stimulus was sufficient to maintain BMC at these sites over the duration of the program. It remains to be seen whether the structural integrity of these sites changes with short duration exercise.

A recent cross-sectional study employed males aged 20-45 years with a weekly running mileage ranging from 5-10 miles/week up to 60-75 miles/week (MacDougall et al. 1992). Bone mineral density for the lower legs was significantly higher in the 15-20 mile/week group than in the control or 5-10 mile/week group. There was also a tendency for a lower bone density of the tibia and spine in the groups whose running mileage exceeded 25 miles/week. When bone cross-sectional area was normalised for body weight there was a tendency for bone area to be larger with increasing mileage, with a significant increase in the 40-50 mile/week group compared to the controls. The authors found that bone cross-sectional area increases disproportionately to bone mineral density, and they suggest the running threshold for the promotion of optimum bone formation is relatively low (i.e., 15-20 miles/week). The reported larger bone widths may be a positive adaptation for mileage above 20 miles/week, and would tend to reduce compression forces on the long bones and provide a protective mechanism against bone injury such as stress fracture.

Increases in cortical bone area have been reported in a group of young male professional tennis players (Jones et al. 1977) as a result of unilateral loading. All players showed a 34.9 per cent increase in hypertrophy on the playing side, with the medullary cavity being commonly narrowed as a result of the thickened cortex. Tennis was also associated with an increase in bone mineral content of the radius in a group of active male players 70-84 years of age, with over 25 years of playing experience (Huddleston et al. 1980). Bone mineral content was greater in the playing arm of tennis players compared to the dominant arm of the non-athletes, but no differences were reported in the non-dominant arms. These results support a specificity of loading concept and absence of transfer effect from dominant to non-dominant limb.

Most information concerning the effects of exercise intervention on bone in males has been derived from investigations of those after the fourth decade of life (Michel et al. 1989). In general, the results from these studies have shown that participation in exercise provides a necessary protective mechanism against age-related bone loss. Michel et al. (1989) assessed bone mineral density of the first lumbar vertebrae using computed tomography, in males aged 50-72 years. Extensive questionnaire information was obtained to estimate the duration and type of exercise required to modify bone tissue. Weight-bearing exercise was defined as the sum of running, aerobic dancing and brisk walking, expressed in minutes per week averaged over the previous twelve months. High correlations were found between weight-bearing exercise and bone mineral density for exercise routines up to 400 minutes per week, in men younger than 65 years of age. Three of the 11 men over the age of 65 years had a reduced BMD despite participating in vigorous exercise. It was concluded that moderate weight-bearing exercise increases lumbar vertebra bone density, however the authors raised the hypothesis that extremely vigorous weight-bearing exercise may be detrimental to bone density in individuals after the age of 65 years.

To lend support to the hypothesis that regular exercise may be an important factor in maintaining skeletal integrity with age, Williams et al. (1984) trained sedentary males (38-68 years) using a running protocol over a 9 month period. Subjects who ran more than 16 kilometres in each month throughout the 9 month period showed a significant increase in BMC of the calcaneus, while those who ran inconsistently, that is less than 16 kilometres in at least one of the months, showed no change. The authors suggested that consistent running in which distance is increased gradually and maintained at a certain level is effective in increasing the BMC of trabecular bone, whereas running that is sporadic and variable has little influence.

Swimming is frequently prescribed as an appropriate activity for improving fitness, without the potential for injury often associated with weight-bearing activities such as running. To investigate the effect of swimming on bone mineralisation, Orwoll et al. (1989) measured radial and vertebral BMD of swimmers who had been swimming for 3 days per week for at least 3 years with non-exercising controls. Radial and vertebral bone density was 12 per cent higher in male swimmers compared to non-swimmers. In contrast to men, the bone density of female swimmers did not differ from the non-swimmers. The positive influence of swimming on bone density in males demonstrates the importance of direct muscle force acting on the bone surface and the associated stimulus of exercise and improved physical fitness as a possible factor in skeletal health. An explanation for the differences between males and females was not provided, however it may reflect differences in hormonal states, as female subjects were either approaching menopause or were postmenopausal.

The majority of the previous literature has indicated that exercise has a specific effect on bone usually in the form of an increase in bone mineral and bone volume. However in a steady state environment, the surface of the bone being remodelled may show a decrease in the balance of bone mineral. This is the basis of the remodelling space theory formulated by Jaworski (1976). The time taken to complete a remodelling cycle is

known as a sigma and approximates 3-4 months for cortical bone and 2-3 months for trabecular bone (Parfitt, 1980). During a sigma period bone volume may have temporarily decreased as a result of the delay between resorption and formation in the normal sequence of bone remodelling. This is known as the resorption space and may account for a decrease of approximately 5 per cent in trabecular BMD and varies from site to site. However this hypothesis is as yet untested in an exercise environment.

Serum Testosterone and Exercise

The replacement of sex hormones such as estrogen has been shown to have a protective effect in reducing the rate of bone loss in postmenopausal women, but has also been shown to increase bone mass if started early enough (Aloia, 1982). Calcium supplementation also provides protection from the accelerated bone loss at menopause, but to a lesser extent than hormone replacement therapy. The valuable information obtained from the numerous studies (Fisher et al. 1986; Dalsky et al. 1988; Snead et al. 1992) concerning sex hormones and calcium replacement therapies in women has stimulated research on the relationship between sex hormones and endurance exercise in males, although inclusion of the measurement of bone density has been neglected.

Early work by Wheeler et al. (1984) was undertaken to investigate whether endurance running in men produced similar hormonal changes to those found in women. The runners who were from local running clubs and ran at least 64 km each week had depressed serum testosterone and prolactin levels. The findings suggest that runners have reduced metabolic clearance and production rates of sex steroids, however the authors were unsure of the mechanisms responsible for these changes. These results are supported by both Hackney et al. (1988) and Griffith et al. (1990) whose findings indicate that endurance trained individuals have significantly lowered serum testosterone and free testosterone levels. As running has become an important recreational and health activity for both cardiovascular fitness and bone health, the results of the latter

investigations tend to suggest the presence of a paradox, as normal testosterone secretion is necessary for maintenance of bone mineralisation in males.

The importance of serum testosterone during growth was elucidated by Krabbe et al. (1979) who found that a sharp increase in serum testosterone production coincided with a growth spurt and an increased bone mineral content between the ages of 13-14 years. This suggests an important role for testosterone in the initiation of both processes and probably the maintenance of bone mineral levels. Krabbe et al. (1984) reported a similar association between the rise in plasma testosterone and the increase in bone mineral content in a longitudinal study of pre-pubertal males, within the age range of 10.7-12.7 years.

The relationship between bone mineral content and serum testosterone was examined further to assess whether androgens can be used as an effective treatment for low bone mineral levels in 29-46 year old males (McElduff et al. 1988). Multiple regression analysis of the data suggested that dominant and non-dominant forearm mineral content was correlated with sex-hormone binding globulin (bound testosterone), although not with serum testosterone. This is supported by a previously described study (MacDougall et al. 1992) whereby serum testosterone concentration was normal for all groups, throughout all running volumes and thus did not appear to have affected bone density.

The Relationship between Bone Strength and Bone Density

The investigation of human cadaver material (Alho et al. 1988; Granhed et al. 1989) has revealed that the strength of bone tissue is highly correlated with bone mineral density, bone mineral content and bone architecture. Alho et al. (1988) determined trabecular BMD of the trochanter and neck of the proximal femur, and of the condyles in the distal femur. A significant relationship was found between the density of the measured sites and the femur's maximal bending strength when a vertical bending load was applied through the femur. The authors concluded that density and strength were evenly

distributed longitudinally in the femur. A relationship between bone mineral content and bone strength has been shown in axial sites. Granhed et al. (1989) reported a significant correlation between the BMC of vertebrae T12 to S1 and their compressive strength. Bone strength is not only a function of its composition but also its geometrical arrangement and the size of its internal architecture, and this is governed primarily by the full loading histories to which the bones are exposed during growth and throughout life (Carter, 1987).

In order to investigate the mechanical and geometrical properties of bone in living tissue, immature pigs were subjected to 12 months of treadmill running (Woo et al. 1981). Biomechanical and biochemical properties were assessed using the right and left femora of the young pigs. The mechanical properties of bone tissue, represented by stiffness and bending stress were similar for the control and experimental groups. Structural properties of the bone showed significant differences between the control and exercised groups. The exercised bone strips were able to absorb more energy before failure, which was due to an average increase of 17 per cent in bone cross-sectional area. Biochemical analysis showed the exercised animals had consistently higher calcium weights indicating an increase in bone volume, as there was no change in bone density of the exercised animals. The results of this investigation suggest that the increase in bone strength following exercise was the result of an increase in bone mass, rather than a change in the composition (density) or geometry of the femora.

The effect of exercise on bone architecture was examined in the rat humeral shaft (Simkin et al. 1989). Two groups of rats were trained to swim for 1 hour a day for 20 weeks, at either a light or moderate training level. The moderate training group had lead weights tied to the roots of their tails. Cross-sectional bone morphology was evaluated as was the ultimate compressive force and stress of the humerus. The results indicated that swimming resulted in higher total subperiosteal and medullary cross-sectional areas compared to the sedentary control rats. The cross-sectional area of the bone cortex was

also significantly greater in the swimming rats resulting from a larger periosteal diameter. A significant increase was found in the ultimate compressive force of the distal humerus in both groups of swimming rats. The authors concluded that swimming increases bone strength as a result of an increase in the cross-sectional area of the bone tissue.

The Deleterious Effect of Exercise and Inactivity

The lack of direct quantitative information concerning the loads produced by physical activity make the prescription of exercise required to optimise the positive effect on bone tissue difficult. The ideal intensity required to stimulate bone mineralisation still remains unanswered, however the results of animal experiments and empirical evidence of young populations suggest caution when associated with high intensity exercise. Several animal studies (Kiiskinen, 1977; Matsuda et al. 1986; Forwood and Parker, 1987) have shown that high intensity training inhibits growth of bone in length and girth. Kiiskinen (1977) subjected young mice to an intensive training program which involved running on a 5 degree inclined treadmill at 30 centimetres per second for 180 minutes a day. Femoral bone volume and strength were significantly decreased after 7 weeks of exercise. However when training was reduced to 30 minutes a day (moderate training) an increase in femoral bone weights was reported. The adaptive responses of immature bones to increased loads was investigated by Matsuda et al. (1986). Young (3 week old) roosters were subjected to intense treadmill running for 5 or 9 weeks in order to observe changes in their tarsometatarsal bones. Reductions in bending stiffness, energy to yield and energy to fracture were observed in both exercise groups as compared to the controls. It was concluded that the high-intensity exercise decreased the material strength of the tarsometatarsal bones during the growth period, by altering the normal processes of calcification and remodelling which are known to influence the stiffness characteristics of bone.

Forwood and Parker (1987) divided pubescent male rats into an exercise and control group to examine the effects of an intensive training program over 1 month on the

mechanical and physical properties of the tibia and femur. Although the mechanical properties of the femur were not affected by the program, the tibia showed a significant reduction in its ability to absorb energy prior to failure. Growth of both the femur and tibia were affected during the exercise program with the tibia showing a decrease in length and the femur a reduction in length and weight. It was suggested that the capacity of the tibia to withstand the intensive loading generated by the exercise program was impaired, due to the accumulation of micro-damage caused by the repeated cyclical loading. Forwood and Parker (1989) demonstrated that as few as 5000 cycles of repetitive loading in-vitro of the tibia and femora of 13 week old rats was enough to create a reduction in the mechanical properties of the bones which was related to diffuse structural damage or microcracking. When microdamage overwhelms the repair of bone, it may accumulate and cause fatigue failures resulting in stress fractures (Frost, 1993). This type of damage may largely result from intensive exercise pursuits (Margulies et al. 1986). Participation in moderate intensity exercise may be below the damage threshold creating a positive remodelling environment and protect younger participants. The line of demarcation between low intensity exercise and moderate intensity exercise needs to be closely defined in order to eliminate the likelihood of providing exercise programs that are of no benefit to bone health. This may be accomplished by closely defining the method used to set the exercise intensity. The degeneration of bone mineral reserves can be observed in studies of reduced weight-bearing, for example spaceflight and immobilisation experimentation.

Spaceflight experimentation allows observation of bone tissue under the influence of a reduced strain environment. Patterson-Buckendahl et al. (1985) observed decreases in bone formation and bone strength in young rats following 7 days of spaceflight, indicating a specific effect on bone due to the weightless environment as body weight remained unaltered. Similar results were reported in an earlier study by Jee et al. (1983) in rats following 18.5 days of spaceflight. Spaceflight decreased the mass of mineralised tissue, and appeared to reduce the number of osteoblast populations but leave osteoclast

numbers unchanged. The results of this study suggested that bone formation may have been inhibited during spaceflight, but resorption remained constant.

Mack et al. (1967) examined astronauts subjected to 4, 8 and 14 days of weightlessness during the Gemini flights. X-rays of the left foot in each astronaut were taken before and after the flights. Bone loss in the os calcis of the command pilot and pilot was between 6-10 per cent during the 4 day and 8 day flight. With the addition of an isometric and isotonic exercise during the 14 day flight, bone loss was reduced in both the command pilot and pilot. Substantial decreases in bone mass are therefore evident when normal mechanical loading is withdrawn.

Several studies have used surgical techniques to investigate the effects of immobilisation on bone tissue (Li et al. 1990; Armstrong, 1946; Sevastikoglou and Larsson, 1977). For example, tenotomy at the knee joint or nerve section of the sciatic nerve in rats resulted in bone loss corresponding to 18 per cent of total femoral mineral content in tenotomized limbs and 12.4 per cent in neurectomized limbs following 10 days postsurgery (Weinreb et al. 1989).

Humans subjected to up to 36 weeks of bed rest had a significantly decreased bone mass in the os calcis by up to 45 per cent (Donaldson et al. 1970) and bed rest for a period of 27 days caused a loss of approximately 0.9 per cent per week in lumbar bone mineral content (Krolner and Toft, 1983). The re-ambulation of subjects in the latter studies restored bone mass to original levels over 36 weeks and 16 weeks respectively. These studies indicate that the response of bones to their mechanical strain environment is a function of the duration, type and intensity of the loading stimulus. By carefully considering the magnitude of the imposed skeletal forces and loading cycles (Whalen et al. 1988), it may be possible to manipulate the loading parameters to stimulate a positive effect on bone mass.

The Relationship between Muscle Strength and Bone Mineralisation

A relationship has been shown between muscle strength, bone density and bone structure. Although the mechanism of this response is poorly understood (Snow-Harter et al. 1990) the functional strain of a muscle tendon pulling at the surface of a bone is believed to be the stimulus required to activate an increase in osteoblastic activity. Men generally have a larger muscle mass than women and therefore muscle tendons in males may exert greater force at their insertion, increasing the size of the underlying structure. Mack et al. (1989) assessed the prominence of deltoid tuberosities using humeral radiographs and bone scans in men and women aged 20-65 years. There was little radiographic evidence to support a relationship between age and the size of the deltoid tuberosity. Deposition of new bone at the deltoid tuberosity region was only slightly correlated with age. When humeral radiographs were reviewed according to sex, 13 of the 14 cases with the most prominent deltoid tuberosities were male. Bone scans of the deltoid tuberosity were also strongly correlated with sex suggesting that a possible relationship between bone size and muscle mass may be found in prominent deltoid tuberosities.

Block et al. (1989) measured the cross-sectional area of the paraspinous muscles of the vertebral column from T12 to L3 in 3 groups of males aged 18-30 years. The groups were made up of weight trainers who trained for a minimum of 12 months, varsity water polo players with an equally vigorous schedule and non-athletes. Each group underwent spine and hip densitometry using computed tomography and dual photon absorptiometry. There were no significant differences between the two athletic groups for any of the bone density measures. The BMD of the two athletic groups was significantly different from the non-athletes, except at the hip in the weight-trainers group. Aerobic conditioning or muscle strength had little influence on the variation in bone density in the athletic groups. The cross-sectional area of the paraspinous muscle showed a moderate correlation with hip bone density in weight-trainers, and was consistently the most important predictor of bone density at the hip and spine, for sub-

groups or the entire population. It was concluded that the size of the vertebral musculature is closely related to the density of the spine.

The effects of a 1 year weight-training program was evaluated in 36-67 year old women who were already participating in an endurance dance program (Peterson et al. 1991). They were compared with other women in the program and with sedentary controls. The weight training-endurance dance group increased in all strength variables, whereas the endurance dance and control group had either smaller increases or decreases in strength. The only significant difference for bone mineralisation appeared in radial BMC, where the endurance dance group was significantly lower than the weight training-endurance dance group. Initial BMD values were correlated with overall strength, although the increased strength found in the weight training-endurance dance group had no effect on BMD. From these results there is evidence of a site specific response in the mineralisation of bone, as strength training of the shoulder and arm flexors and extensors was found to increase in BMD at the radius. No other response was evident, although the load-bearing exercise was sufficient to maintain BMD.

Pocock et al. (1989) assessed the relative importance of muscle strength, physical fitness and body mass index on the age-related decline in BMD. In this investigation healthy females aged 20-75 years participated in an assessment of BMD in the lumbar spine, proximal femur and forearm, and these measures were related to muscle strength of the quadriceps, bicep and grip strength. Muscle strength was found to be an independent predictor of BMD at all 3 sites in the proximal femur as well as in the lumbar spine and forearm. Age was not an independent predictor of bone density in the proximal femur of the entire group. The authors suggests that the reduction in femoral neck bone mass with increasing age may be due to age associated changes in mechanical loading of the skeleton and not a consequence of aging. Therefore this may be preventable with increased mechanical loading and physical activity programs.

The Relationship between Cardiovascular Fitness and Bone Density

A commonly accepted outcome and requirement of organised physical activity programs is an increase in cardiovascular fitness, and there is evidence that improvement in skeletal health following exercise may be a function of improved cardiovascular fitness. For example, BMD of the lumbar spine and femoral neck in women aged 20-75 years was correlated with their level of physical fitness (Pocock et al. 1986). Predicted maximal oxygen uptake was measured from a submaximal stress test using a bicycle ergometer. Similarly, Chow et al. (1986) found that physical fitness measured through predicted maximal oxygen uptake (VO_2max) was significantly correlated with the calcium bone index measured in the lumbar vertebrae and proximal femur of postmenopausal women aged 55-75 years.

In contrast to the latter method, Bevier et al. (1989) measured oxygen consumption directly during maximal work in healthy men and women aged 61-84 years and failed to find any correlation between aerobic capacity and bone density measured at the lumbar spine and mid-radius. Similarly, Block et al. (1989) examined a group of young highly trained aerobic athletes and found no linear association between aerobic capacity (VO_2max) and bone density of the spine or hip. This discrepancy may be explained through important differences in experimental procedures used to predict oxygen uptake which may introduce errors of up to $\pm$15 per cent (Davies, 1968).

An early investigation involving males (Dalen and Olsson, 1974) reported an 11 per cent increase in aerobic capacity following a 3 month exercise program. The exercise program maintained BMC of the exercise group, however there was no association between cardiorespiratory fitness and BMC. The mechanical stimulus provided by the weight-bearing exercise associated with the aerobic activity may not been sufficient to increase BMC or to create a physiological response at the local tissue level that would increase bone turnover.

An investigation of postmenopausal women aged 55-70 engaged in a 9 month endurance training program (Dalsky et al. 1988) reported significant improvement in the BMD of the lumbar spine of the exercise group. However they failed to observe a significant relationship between the changes in bone mineral density at the lumbar spine and changes in aerobic capacity (VO_2max). Chow et al. (1987) measured cardiorespiratory fitness and bone mass of postmenopausal women following a year long aerobic exercise program. Maximal oxygen uptake, a measure of aerobic capacity was not determined by gas analysis but predicted from the maximum workload. The exercise program resulted in an increased bone mass (calcium bone index) which showed a significant relationship with the improvement in aerobic capacity (predicted VO_2max). This may again suggest that the errors induced by a prediction of cardiorespiratory or cardiovascular fitness have considerable bearing on the results of these studies.

Biochemical Indices of Bone Turnover

Biochemical bone markers are used as indices of bone resorption and formation and the two most widely used are urinary hydroxyproline and serum osteocalcin (Brown et al. 1984). Hydroxyproline is an amino acid found almost exclusively in collagen, and the peptides containing hydroxyproline originating from the degradation of collagen are found in urine (Sambrook, 1991). As such the measurement of urinary hydroxyproline is particularly useful in monitoring the treatment and progress of metabolic bone diseases such as osteoporosis in which bone resorption is a significant factor.

Osteocalcin (bone gla-protein = BGP)) is a single chain amino acid of low molecular weight, which has three residues of gamma carboxyglutamic acid (gla), a calcium binding amino acid in the presence of vitamin K. In contrast to gla, which is found in a variety of mineralised tissues, osteocalcin has only been found in bone and in small amounts in tooth dentin. Several experiments have shown that osteocalcin is synthesised by the osteoblasts in bone (Lian and Friedman, 1978; Nishimoto and Price, 1979), however the physiological role of osteocalcin is still unknown (Delmas, 1988). It has

been estimated that approximately 15 per cent of the osteocalcin synthesised is released into the circulation where it can be measured by radioimmunoassay, and the remaining 85 per cent is incorporated into bone, where it will bind to the mineral (Price and Nishimoto, 1980). However these figures apply to bovine and not to human osteocalcin.

Most radioimmunoassays are developed with antisera raised against bovine osteocalcin, and this is used to cross react with human osteocalcin (Delmas et al. 1983). These assays provide a normal mean value of serum osteocalcin in adults ranging from 4.2 to 7 ng/ml, with individuals ranging from 2-13 ng/ml. Cole et al. (1983) conducted an extensive study on serum osteocalcin concentrations in normal infants, children and adolescents. The mean osteocalcin in infant boys (16 ± 2.3 ng/ml) was not significantly different than in girls (14.5 ± 2.1 ng/ml). Osteocalcin in boys began to rise at about age 12 years, and peaked 2 years later (mean 39 ng/ml), and then declined toward adult levels at age 18 to 20 years. Elevation of osteocalcin was evident in girls by age 10, and was less marked than in boys (mean 26 ng/ml), and declined toward the low adult range earlier. Catherwood et al. (1985) undertook a radioimmunoassay based on antiserum raised against a small fragment of human osteocalcin in healthy male and female subjects, aged 23-91 years. The use of this antiserum tended to provide much higher values, with a mean of 15 ng/ml and a wide individual range from 0-40 ng/ml.

The biochemical bone markers urinary hydroxyproline and serum osteocalcin have largely been used to assess the rate of change in bone turnover in aging women and men (Delmas et al. 1983; Epstein et al. 1984; Kelly et al. 1989; Resch et al. 1992). The results of these studies suggest that there is a gradual decline in skeletal mass with age in women as a result of a decline in bone formation rate, and an accompanying increase in bone resorption. Serum osteocalcin values were found to be higher in women compared to a small steady increase in men. The rise in serum osteocalcin in men was most

pronounced between the eighth and ninth decades, when osteoporosis is most common in males which coincides with elevated urinary hydroxyproline.

Biochemical markers of bone metabolism have also been used to assess the effect of immobilisation and weightlessness on bone resorption (Donaldson et al. 1970; Patterson-Buckendahl et al. 1985; van der Wiel et al. 1991). In an early investigation (Donaldson et al. 1970) 3 healthy adult males (ages 21-22 years) were restricted to complete bed rest for periods of 30-36 weeks. At the end of this period there was a large decline in the bone mineral content in all 3 subjects, however urinary hydroxyproline was only mildly elevated. Upon re-ambulation bone mineral accumulated at a similar rate to the rate at which it was lost and urinary hydroxyproline levels fell to pre bed rest levels. A recent study by Van der Wiel et al. (1991) investigated the early onset of increased bone resorption in 9 women (mean 39.2 years) and 5 men (mean 45.6 years) for a period of 10 days. The subjects had been hospitalised due to lumbar disc protrusion. Urinary hydroxyproline increased significantly after 4 days (30 per cent), reached a peak after 10 days (70 per cent) and slowly returned to baseline values after 6 weeks of mobilisation. The results show that the onset of increased bone resorption occurs very early during immobilisation due to the decreased strain in bone, indicating the importance of remaining active.

Patterson-Buckendahl et al. (1985) measured the osteocalcin content of bone extracts from the vertebrae and humerus and from the serum in young rats following 7 days of spaceflight (F). Serum osteocalcin was decreased by comparison with ground simulation controls sacrificed after 9 days (S). It was concluded that the source of serum osteocalcin is new synthesis by bone cells, and that the decreased levels of osteocalcin may represent decreased osteoblastic activity associated with reduced skeletal growth during spaceflight.

There is a paucity of information on the use of biochemical parameters in assessing the response of bone to exercise. McCarthy and Jeffcott (1992) compared the effects of a 14 week period of exercise on bone metabolism in young male and female horses aged between 13 and 14 months. The exercise program involved 9 weeks of trotting and cantering on a treadmill with a 3 degree incline. Over the final 5 weeks the horses were exercised at near maximal speeds with no incline. Bone mineral content was determined by single photon absorptiometry at the mid-metacarpus in both left and right limbs and serum osteocalcin was measured at weeks 8 and 14. BMC increased significantly in the exercising horses by the end of training and only increased slightly in the non-exercised animals over the same period. Serum osteocalcin increased significantly in the non-exercising horses between weeks 8 to 14, whereas the exercising group experienced no significant increase in this time. The osteocalcin level in the exercising group at week 14 was lower than in the non-exercising controls, although it was not significant. The results of this study indicate that the increase in BMC was due to an adaptation to the loads applied by the training program. The approaching significance of serum osteocalcin in the exercising group may indicate that bone remodelling was occurring, while the non-exercisers continued the normal modelling processes associated with growth.

Kelly et al. (1990) examined women aged 19-83 years in order to find a relationship between somatomedin-C, physical fitness and bone density. A secondary purpose to the experiment, relevant to this investigation, was the use of urinary hydroxyproline and serum osteocalcin to assess biochemical indices of bone formation and resorption. Bone density was measured at the lumbar spine, femoral neck, greater trochanter, and Ward's triangle using dual photon absorptiometry. Single photon absorptiometry was used to measure the distal forearm. No correlation could be found between serum osteocalcin and any of the bone density measures, however negative correlations were found between hydroxyproline and the lumbar spine, femoral neck and the distal radius. The decline of bone density with age was associated with increased bone resorption which

may account for the negative correlation in this study. No information was provided on any possible relationship between bone resorption and formation parameters and physical fitness.

Measurement of Bone Density

The earliest available, accurate and precise methods of assessing bone mass were through the use of single-photon absorptiometry (SPA). This involves passing a highly collimated mono energetic beam of photons (using ^{125}I as the source) across a limb, and monitoring the transmitted radiation with a sodium iodide scintillation detector (American College of Physicians, 1984). The technique allows the calculation of total bone mineral content in the path of the beam, measured as grams per centimetre. SPA is limited to peripheral sites and cannot measure bone density of the hip or spine or discriminate between cortical and trabecular bone (American College of Physicians, 1987). The technique exposes subjects to a relatively low radiation level (20-100 μSv) with accuracy error being approximately 4-5 per cent and precision error 1-2 per cent in clinical settings (Melton et al. 1990). Many early exercise intervention studies used this method for observing changes in the BMD of the forearm (Sinaki et al. 1974; Krolner et al. 1980) and the method is still used as a valid measurement of BMD (Nelson et al. 1991; Virvidakis et al. 1991).

Techniques for the measurement of bone mineral density and content developed further with the use of dual-photon absorptiometry which emits photons at two different energies, using ^{153}Gd as the source. Direct measurement of bone mineral density in $g.cm^{-2}$, and scanning of the hip and spine is allowed, however the technique cannot distinguish between cortical and trabecular bone (Gotfredsen et al. 1984). Accuracy error is 3-6 per cent for the spine and 3-4 per cent for the hip. Precision error is 2-4 per cent and 4 per cent for the spine and hip respectively and radiation level is 50 μSv for a regional scan (Melton et al. 1990). This procedure is still commonly used especially in studies designed to investigate the rate of change of BMD (Drinkwater et al. 1991; Harris

and Dawson-Hughes, 1992). The method is limited as a useful screening device for use with large numbers of people due to the length of time taken to complete a scan. For example a scan of the L1-L4 region takes approximately 45 minutes and involves an extended period of radiation exposure (Kelly et al. 1988)

Dual energy x-ray absorptiometry (DEXA) replaces the radioactive ^{153}Gd used in dual photon absorptiometry and reduces by 50 per cent the precision error of measurement *in vivo*. In addition the time of the scans (Mazess et al. 1989) is significantly reduced. It must be recognised that precision results *in vivo* are not always directly reflected *in vitro*. One DEXA scanner gives a 0.5 per cent precision on spine phantoms, yet on normal subjects it has a precision of 1.4 per cent on the spine and 2.3 per cent on the femur (Kelly et al. 1988) which is due to the presence of lean tissue over the site to be scanned. Patient re-positioning is a factor that is especially important in achieving good precision of measurement, considering the range of possible trunk, leg and foot orientations that may be encountered during scanning. Wilson (1991) found the predicted precision of bone mineral density measurements of the femoral neck and Ward's triangle was approximately 3 per cent if the re-positioning of the foot or leg was inconsistent. They concluded a measurement precision of 3-5 per cent is adequate to see if an individuals' BMD or BMC has deviated significantly from the mean of a normal population. Nuti et al. (1992) evaluated the BMD of 330 postmenopausal women, where 267 women in this sample were affected by postmenopausal osteoporosis. It was considered that in total body densitometry, DEXA was a reliable tool in determining conditions associated with bone loss, as it avoided the methodological problems of positioning that affect forearm and femoral measurements. This is in agreement with Laitinen et al. (1991) who used DEXA on a large population of Finnish women because of its reduced scanning time, less radiation exposure, better resolution and precision. DEXA has also been successfully used to measure BMD in athletic or exercising populations (Myburgh et al. 1990).

Summary

Studies of growth related changes in bone density now seem to agree that peak bone mass is achieved in the third decade of life, and it is becoming increasingly important to investigate whether it is possible to modify bone density above peak bone mass levels following skeletal maturity. At this age it may be easier to implement lifestyle adjustments such as a weight-bearing exercise program. Information gained from such studies may facilitate the use of exercise as an important intervention in those who are susceptible to bone loss disorders later in life. The studies that have been reviewed in this chapter have largely concentrated on physically active aging male and female subjects. The response of younger populations and especially young male subject's to physical activity have not been addressed. Two studies that have been reported extensively in this review have investigated the effects of an exercise intervention on a young male population but have utilised an intensive exercise regime. The importance of mechanical and physiological adaptation to a lifestyle stimulus such as moderate intensity exercise has yet to be investigated in a young male population. The research design to be used in this training intervention study is different to that previously used in a similar population. The chapter to follow will outline such an intervention in a young male population in order to extend current findings in the literature.

CHAPTER 3

METHODS

Subjects

Twenty-six sedentary male subjects aged 20-27 years (average 22.2 years) volunteered from the student body at the University of Wollongong for participation in this study. The subjects were recruited from responses to fliers posted around the campus. In order to participate in the study subjects must not have undertaken more than 1 hour of aerobic exercise per week. These subjects defined as sedentary were identified with the use of an activity questionnaire (Appendix III). A medical questionnaire (Appendix II) was used to identify participants with conditions known to be associated with bone loss. Potential subjects with musculoskeletal or endocrine disorders or any person on medication or drugs that are known to effect bone metabolism were excluded from the study, as well as those with known cardiovascular disease. Cigarette smoking or excessive alcohol intake (>3 drinks per day) were also grounds for exclusion from the study. Sixteen subjects were assigned to the exercise program and the remaining ten were assigned to the control group. The greater number of subjects in the exercise group was to allow for subject attrition over the sixteen week intervention period.

Anthropometry

Anthropometric measures were taken to estimate the effectiveness of the exercise training program and to examine the relationship between height, weight, and bone mineral density and mass. A number of studies have found correlations between height, weight, BMD and BMC (Glastre et al. 1990; Kroger et al. 1992; Miller et al. 1991; Thomas et al. 1991). Therefore it was considered important to determine if any possible decreases in weight brought about by the running program would show an association with BMD and BMC. Methods for obtaining anthropometric data were based on standard techniques described by the International Biological Programme (Weiner and Lourie, 1969). Height was measured to the nearest centimetre (Holtain Ltd. Crymych Stadiometer) and weight

recorded to the nearest 20 grams (AND Electronic scales-FW 150K), both measurements requiring shoes and socks to be removed.

Fitness Testing Procedure

To overcome anxiety on the day of testing, a day was set before the maximal exercise test for the participants to visit the human performance laboratory and become familiar with the treadmill and its operation. Testing was carried out in the morning, following a night of approximately eight hours sleep by the subject. A number of preparatory requirements to be followed prior to testing were issued to subjects. These included: avoidance of exhausting muscular work on the preceding day; a request to avoid participation in exercise three hours prior to the test; and to refrain from ingestion of any food, tobacco or alcohol for three hours prior to testing.

The Polar PE 3000 sportstester belt (Validation; Appendix VII) was placed in the middle of the sternum, running parallel with the fifth costal cartilage, and the receiver watch strapped to the treadmill in front of the subject. Participants were then required to take part in a 10 minute stretching routine of the muscles of the lower limbs in order to increase blood flow to the peripheries, and to reduce the possibility of muscular injury during the maximal exercise test. The subject was then asked to straddle the level treadmill belt (Quinton Instruments model 18-60-1) and once a heart rate signal from the sportstester was obtained, they were requested to begin running at the set speed of 7.5 kilometres per hour. Following each minute of the maximal exercise test the inclination of the treadmill was increased by 2 per cent until the point of exhaustion, at which time the maximum heart rate was recorded and the test terminated. Exhaustion was defined as the time when the subject could not continue exercising and was required to assist himself with the support bars on the treadmill. Following completion of the test a cool down period was provided. This involved walking on the treadmill, set at a speed of 3 kilometres per hour for 3 minutes, followed by light stretching exercises in order to prevent blood pooling in the lower extremities.

Analysis of the recorded data involved downloading the stored heart rates into an ASCII file using the Polar PE3000 sportstester interface and computer analysis package. ASCII file data was then imported into the Sigma Plot graphics program (Jandel Scientific) to enable the area under the curve (sum of the heart rates) to be calculated. The summing of heart rates for each minute of the maximal treadmill test was termed cumulative heart rate. Cumulative heart rate was used as one measure of subjects' cardiovascular fitness, as submaximal heart rate is known to decrease with an increase in fitness (Saltin et al. 1976). Heart rates from the initial and final maximal treadmill tests were matched minute for minute. This enabled a comparison of cumulative heart rates for the same absolute workload. For example, if subject A ran for 10 minutes in his initial maximal treadmill test and 12 minutes in his final test, then cumulative heart rate in the final test was matched up to the 10th minute.

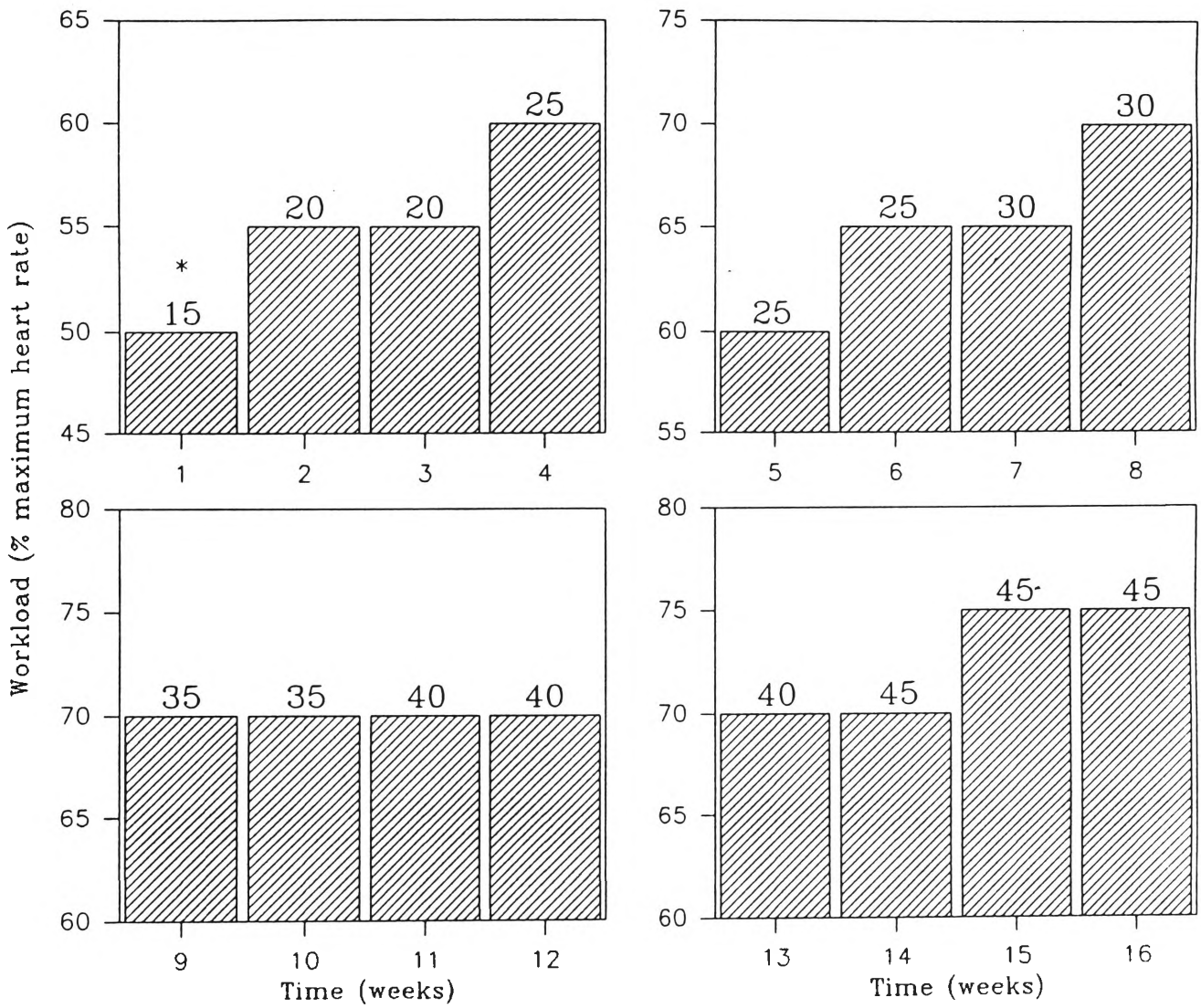
As shown in previous training studies (Hardman et al. 1986; Boobis et al. 1984), an increase in time to exhaustion on the treadmill is an adaptation to endurance training. Therefore time to exhaustion during the treadmill test was used as the second measure of subjects' cardiovascular fitness.

Previous investigations have predicted cardiovascular fitness levels from submaximal exercise protocols (Pocock et al. 1986). However, the prediction of maximal oxygen uptake and maximal heart rate from the submaximal heart rate response has been repeatedly criticised for its lack of accuracy (Davies, 1968; Rowell et al. 1964; Taylor et al. 1963). Estimation of maximal heart rate may also be affected by the emotional state or degree of excitement of the subject, the degree of physical conditioning, the time after a previous meal, degree of hydration of the subject (Buskirk et al. 1958) and any alterations in ambient temperature (Brouha et al. 1961). To avoid these limitations a method was adopted in this study that required subjects to run to exhaustion on the treadmill and the investigator recorded and utilised their maximum heart rate, as a more direct measure of fitness.

Exercise Training Program

The exercise program (Figure 1) required participants to run 3 days per week, for 16 weeks (48 sessions), a period of time that has previously been shown to induce a change in bone density (Margulies et al. 1986) in sedentary populations. The exercise intensity was determined from a percentage of the maximum heart rate and participants were required to run within a 5 bpm range above or below this set percentage. For example, a subject with a maximal heart rate of 200 bpm running in week 5 of the program was required to run at 60 per cent of maximum heart rate. The training zone would then be set between 115-125 bpm. To remain within their individually set training zones, running speed was increased or decreased in relation to their heart rate. Therefore it was important that subjects could accurately monitor their heart rate over the course of the running program. Instruction on how to record and monitor the radial pulse during the supervised runs was provided. At predetermined points along the running track the subjects' counted their pulse over a six second period, and multiplied this score by ten, to obtain an estimate of their heart rate (bpm), and to stay within their training zones. Maximal treadmill tests were undertaken each month by the exercising group and the results used to adjust the training intensity. This procedure was implemented because it has been found that the most appropriate and feasible control of training occurs when work rates are adjusted regularly, in parallel with improvements in physical fitness (Nordesjo 1974; Saltin et al. 1976).

The link between exercise duration and modification of bone density is through repetitive loading of the skeletal system (Williams et al. 1984; Dalsky et al. 1988) and it was acknowledged that additional weight-bearing exercise undertaken by the experimental and control groups may confound the results of the running program in this investigation. Therefore during the course of the investigation both experimental and control groups were required to keep a diary of all activity undertaken, to quantify the amount of loading created by activity, apart from that of the running program itself. It was stipulated that the records must be as accurate and specific as possible and the diary must be carried at all



* Denotes the duration (minutes) of the 3 runs during that particular week.

Figure 3.1. The 16-week running intervention program for members of the exercise group.

times. The information recorded in the diary was used to estimate the number of hours of additional activity over the duration of the investigation.

When subjects were unable to attend the supervised runs, they were required to complete a make-up run. The participant was informed of the duration and intensity of the run they had missed. The make-up run was completed unsupervised and in the subjects' own time. The time of the run was recorded in the diary and used at the end of the 16 weeks to determine the total participation of each subject over the 16 weeks. Of the sixteen young males assigned to the exercise group, 12 (75 per cent) completed the 16 week running program. One left in the second week due to job commitments, two in the third and fifth week because of insufficient time and one in the fourth week as a result of knee pain. All the controls completed the follow-up tests following the 16 week exercise program.

Bone Composition Protocol

The measurement of bone mineral density and bone mineral content was undertaken by dual-energy x-ray absorptiometry using the Norland XR-26 bone densitometer. Prior to the measurement of subjects in the study a pilot project was conducted to assess the effects of variation in tissue thickness on bone mineral density and bone mineral content, using a cadaver section of the proximal femur. Two studies have shown that tissue thickness has a significant effect on bone mineral density and bone mineral content using dual energy x-ray absorptiometry (Mazess et al. 1989; Arai et al. 1990). The cadaver was supplied by the University of Wollongong Anatomy Laboratory. The proximal femur was used as it contained the three areas to be examined in this investigation, the femoral neck, greater trochanter and Ward's triangle. It was not possible to repeat this procedure using cadaveric material from the lumbar spine.

The sites to be examined on the femur were covered by muscle tissue, adipose tissue and skin. The anterior and posterior depths of muscle, adipose tissue and skin was 3cm and

6 cm respectively over the femoral neck. The cadaver section was placed in position on the Norland XR-26 Bone Densitometer and a series of five scans were taken of the area. A mark was placed on the skin to enable accurate positioning of the laser for each scan. Following the completion of these scans a pin was placed in the centre of the mark in order to retain this position once the tissue was removed. This procedure allowing the laser to be placed over the femoral neck in an identical position. The cadaver section was then taken back to the anatomy laboratory and all tissue around the femoral neck, greater trochanter and Ward's triangle was removed. This began with the subcutaneous fat on the anterior and posterior aspect of the cadaver followed by the anterior muscles rectus femoris, sartorius, iliopsoas, adductor magnus and pectineus. Posteriorly, the tensor facia latae, gluteus maximus, medius, minimus, and quadratus femoris were resected. At this point the femoral neck, greater trochanter and Ward's triangle were visible but covered by the ilio-femoral, ishio-femoral and pubo-femoral ligaments. The cadaver section was then rescanned five times using the same procedure.

Paired t-tests were completed on the measurements for BMD and BMC of the femoral neck, greater trochanter and Ward's triangle to assess differences following removal of the tissue. A significant difference was found in the BMD of the femoral neck ($P < 0.05$). Results of this procedure are presented in Figure 3.2.

Due to the longitudinal nature of this investigation it was necessary to examine the reliability of the Norland XR-26 bone densitometer using repeated scans, and to develop a reliable method for re-positioning subjects to improve the reliability of scans over the 16 week period. It was also important to monitor the short term system precision of this instrument through repeated scans of the Norland Anthropometric Spine Phantom with a known BMD and BMC. This provided a coefficient of variation (CV) which was used to determine error (imprecision) of the BMD and BMC measurements.

As subject positioning was a potential source of error, repeat scans for bone mineral density and bone mineral content were taken of the lumbar spine and proximal femur on 5 volunteers to develop a reliable method for the re-positioning of subjects. The scan of the lumbar spine required the subjects to have a cubed block placed under the lower legs, so that the lumbar spine was flat on the scanner table. To obtain the same position for the follow-up scan a goniometer was used to measure the angle at the hip. The anchored arm of the goniometer was positioned through the mid-axilla region and the movable arm positioned through the lateral femoral epicondyle with the axis of the goniometer on the greater trochanter. The scans taken at the three sites on the proximal femur required the subjects to remove their shoes and place their feet in the trapezoid foot block. This created a small amount of inward rotation at the hip. Measurement of the amount of inward rotation was not required as the feet were securely fastened into the foot block. Between scans the subject temporarily left their position on the scanner table, then returned to be repositioned by the operator for the second scan, which was used to check the repeatability of subject positioning. Correlation coefficients (r) for the reliability of repeat scan measurements at all sites are summarised in Table 1 and all raw data is presented in Appendix XXII.

Table 1. Correlation coefficients (r) for repeat scan reliability using the Norland XR-26 bone densitometer for the sites on the proximal femur and lumbar spine (L2-L4).

	Femoral Neck		**Trochanter**		**Ward's Triangle**	
	BMD	**BMC**	**BMD**	**BMC**	**BMD**	**BMC**
r	0.996	0.997	0.993	0.545	0.958	0.959

	L2		**L3**		**L4**		**L2-L4**	
	BMD	**BMC**	**BMD**	**BMC**	**BMD**	**BMC**	**BMD**	**BMC**
r	0.816	0.966	0.966	0.953	0.906	0.944	0.932	0.939

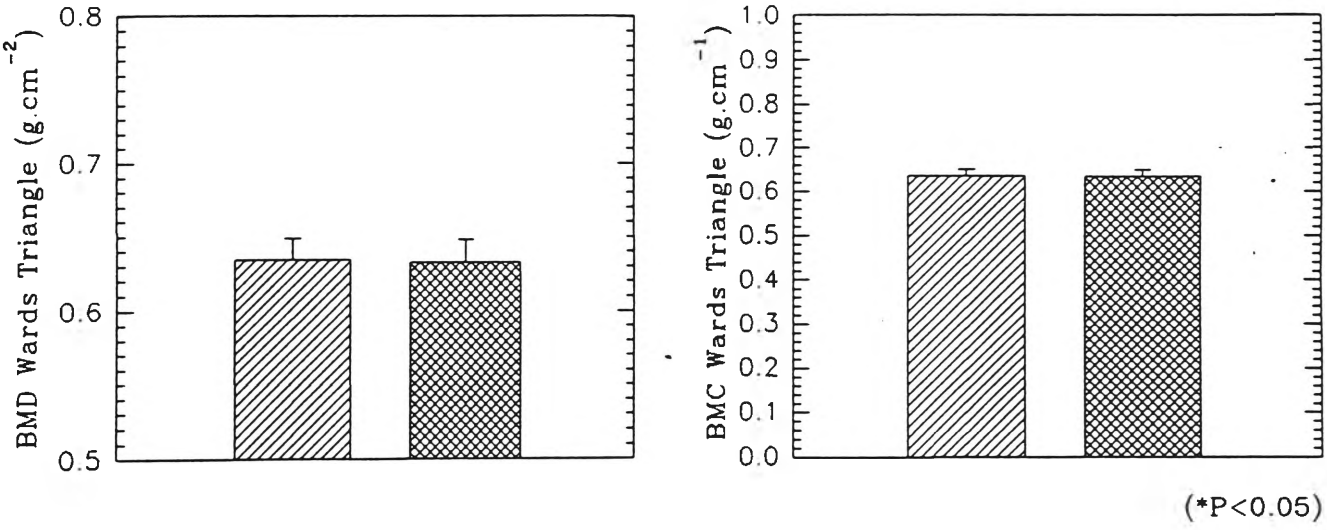
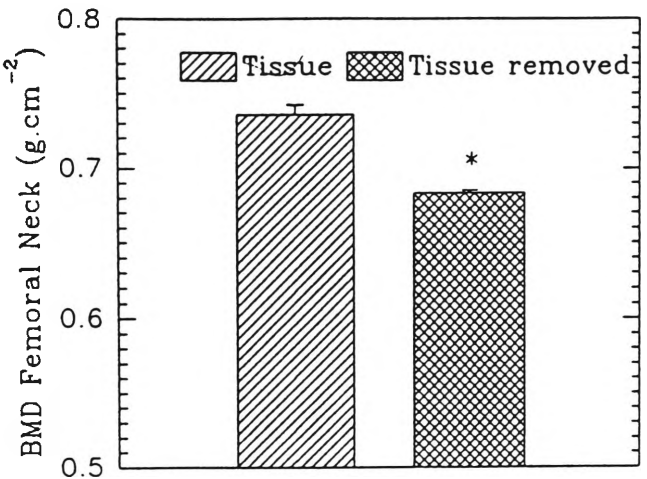
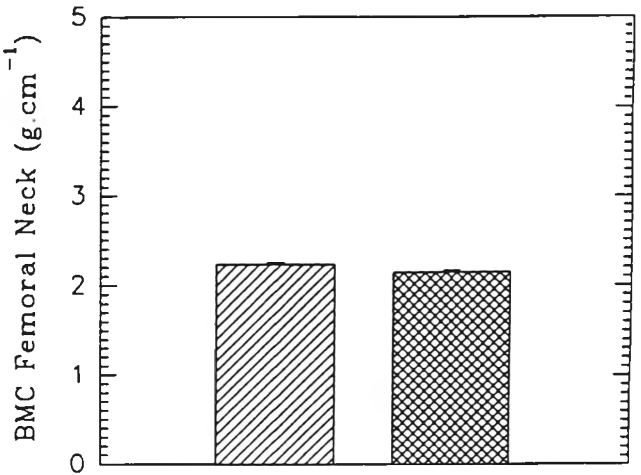
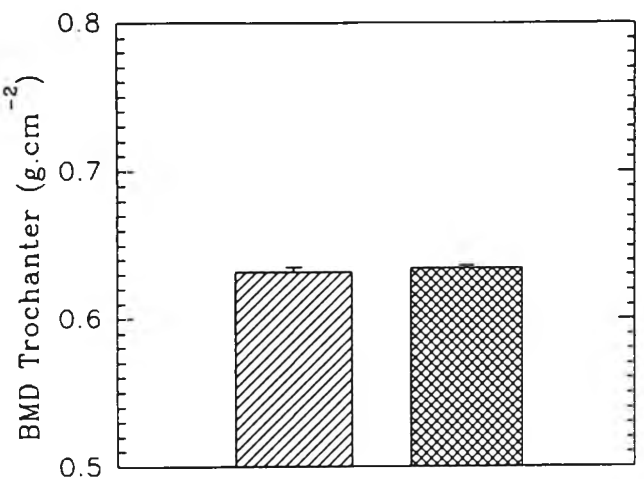
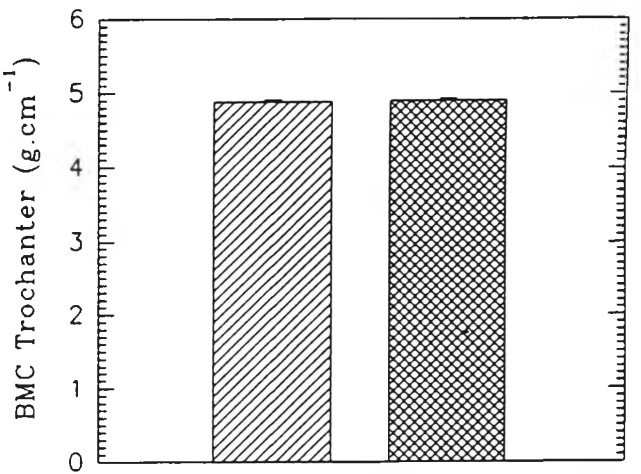


Figure 3.2. Effects of variation in tissue thickness on bone mineral density ($g.cm^{-2}$) and bone mineral content ($g.cm^{-1}$) using a cadaver section of the proximal femur.


Tissue
Tissue removed
BMD Femoral Neck (g.cm^{-2})
0.8
0.7
0.6
0.5
*


BMC Femoral Neck (g.cm^{-1})
5
4
3
2
1
0


BMD Trochanter (g.cm^{-2})
0.8
0.7
0.6
0.5


BMC Trochanter (g.cm^{-1})
6
5
4
3
2
1
0

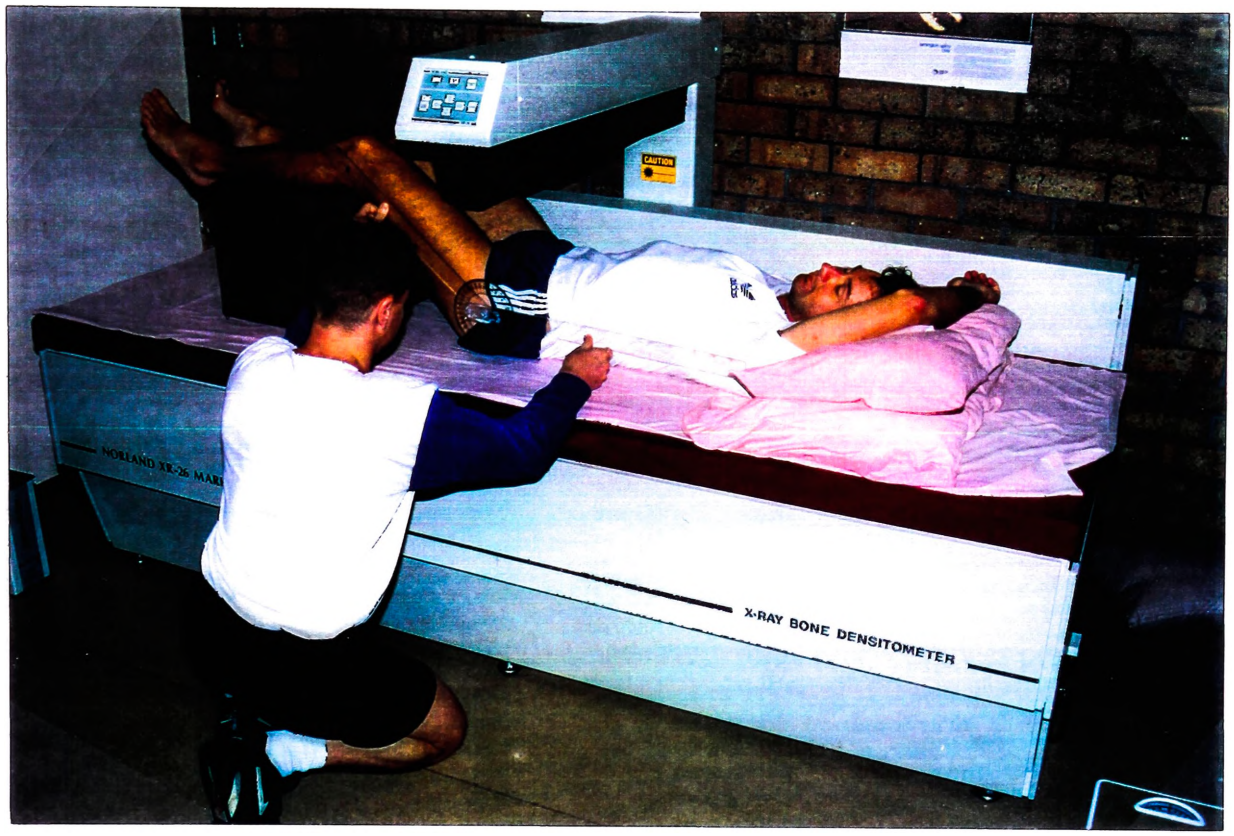


Figure 3.3. (a). The position of subjects for the anterior-posterior scan of the lumbar vertebrae using the Norland XR-26 bone densitometer .

(b). The position of subjects for the scan of the proximal femur using the Norland XR-26 bone densitometer.

Instrument Calibration

The precision of the Norland XR-26 bone densitometer was tested using a modification of the standard procedure recommended by the manufacturer. Norland factory values of the calcium sulfate spine phantom for BMD was 0.855 $g.cm^{-2}$ and for BMC was 33.64 $g.cm^{-1}$. In this investigation the spine phantom was scanned 10 times with the phantom repositioned on each occasion. The CV of these results was calculated using the equation in Appendix X, and the CV or precision error for BMD and BMC was 0.58 per cent and 1.33 per cent respectively.

Prior to each measurement session an automatic calibration procedure was performed to assess and maintain measurement precision and accuracy of the Norland XR-26. Full explanation of the calibration procedure is found in Appendix IX.

Experimental Protocol

Scanning of the lumbar spine was conducted first, followed by the proximal femur, with scanning time being approximately 30 minutes. Measurement of the lumbar spine in the anterior/posterior direction included the bodies and spinous processes of the vertebrae, but excluded the transverse processes. All metals were removed from the subjects, metals are seen as high density areas, which can cause false readings. The subject lay supine with the right side of the body adjacent to the scanner backrest, and a cubed-block leg rest was placed under the subject's legs to reduce the lumbar lordosis. The angle at the hip was measured as described earlier (p.43). A low power Helium-neon laser, visible as a red dot was then projected onto the subject, to facilitate measurement of the start and end points of the scan area. The scan area was delineated between the start point, 3cm below the xiphiod process and the end point, 3cm below the iliac crest. During scanning, which lasted for an average of 6 minutes, the subject was instructed to remain as still as possible. When the scan was complete the investigator used the generated computer image of the lumbar vertebrae to define the area for which BMD and BMC were to be measured.

Measurement of BMD and BMC of the proximal femur was taken from the dominant leg of the subject. The subject lay supine on the scanner table with the right side of the body adjacent to the scanner backrest. A trapezoid shaped foot block was used to create a slight amount of inward rotation at the hip. The greater trochanter was palpated, and used to estimate the centre of the femoral neck, which was then marked by positioning the red dot from the laser over this area, to define the start point of the scan. The process began with a brief 2 minute *scout* scan over the area of the femoral neck to generate a computer image of the region scanned. This image was then used to more accurately define the *measurement* scan area on the proximal femur. Following the *measurement* scan, which lasted for an average of 4 minutes, the generated computer image was used to define the area on the proximal femur for measurement of BMD and BMC.

Following the 16 week exercise program measurement of BMD and BMC of the same sites were repeated using the procedures described earlier. When the scans were repeated, the Norland software provided a comparison image of the subjects' first scan This allowed the operator to define the area of measurement for the second scan from the comparison image of the first scan. The software also identified if the subject had a previous scan by recognition of the subject's name, and used this information to automatically define the area of measurement. This allowed for greater accuracy when the subjects were required to be re-scanned following the 16 week program.

Biochemical Analysis

Biochemical assays were conducted at Sugerman's Pathology, Sydney by the investigator, under the supervision of the Pathologist on duty. In order to minimise sources of error it was necessary to visit the pathology laboratory and become familiar with techniques involving Radioimmunoassay and Photometric analysis. This required competence in the use of a gamma counter and spectrophotometer, and accuracy in the pipetting of reagents, as this is the main source of error during assay preparation.

Urinary Hydroxyproline Procedure

A urine sample was collected from each subject using a set of instructions provided by Sugerman's Pathology (Appendix XI) for the collection procedure known as a fasting 2 hour procedure or "spot technique" (Nordin et al. 1976).

Urinary hydroxyproline was measured in umol/mmol creatinine with a reference range of 0-28 umol/mmol creatinine for the normal population. Hydroxyproline, occurring in the urine is mainly peptide bound and this peptide bound form was bound to a strong acid cation exchange resin. By washing the resin with distilled water, several components which interfere with the determination were eliminated. The peptides bound onto the resin were then hydrolysed by elevating the temperature to 100°C for approximately 16 hours. After the elution of the hydroxyproline from the resin, the amino acid was oxidised forming a pyrrole derivative. This was coloured by adding Ehrlich's reagent and quantitatively determined by photometric analysis (Varian DMS 80 UV-Vis Spectrophotometer). The Hypronosticon kit provided by Organo-Technika Catalogue No. 2768004 contained the working reagents (Hypronostican manual, 1989). All samples were analysed in the same batch, to exclude any batch to batch variation.

Serum Osteocalcin Procedure

The determination of osteocalcin required the collection of a 50uL serum sample to assay the specimen in duplicate. An area of at least one and a half inches in all directions from the intended site of venipuncture was prepared. The area was scrubbed with 15 per cent aqueous (non-alcoholic) soap or detergent solution to clean away fat, oils, dirt, skin cells and other debris. This was followed by a 70 per cent isopropyl alcohol sterile swab, moving from the venipuncture site outward. A sterile needle was used immediately and the blood drawn. Blood was then collected in a SST Vacutainer tube and the blood allowed to clot. The sample was then centrifuged (IEC Centra-3C, England) for 15 minutes at 3000rpm to separate the serum from the cells. Samples were frozen at -20°C or below immediately following separation, while awaiting measurement.

Serum Osteocalcin was analysed through radioimmunoassay techniques (LKB Gamma Counter, 1260 Multigamma) utilising the Nichols Institute Diagnostics Human Osteocalcin Radioimmunoassay Kit Catalogue No.#40-2225, Item #36B-2225. The assay procedure required the concentration of unlabelled hormone (antigen) to be quantitated by its competition with a trace amount of radio-labelled antigen for specific antibody binding sites. With increasing concentration of unlabelled substance, decreasing concentration of the label were bound to the antisera. Thus, a dose-response relationship was drawn by measuring the concentration of bound radio-label resulting from competition with concentrations of known antibody for a limited number of antibody binding sites. Sera containing an unknown concentration of the antibody was quantitated by comparison with the dose response curve. The Diagnostic kit used purified human osteocalcin as standard and tracer. After a three hour incubation with a specific rabbit antiserum, a solid phase anti-rabbit (donkey) coated cellulose suspension was used to separate the bound from free osteocalcin (Nichols Institute Diagnostics, Directional Insert 1992).

Nichols Institute Diagnostics reports precision and reproducability of the Human Osteocalcin RIA. The coefficient of variation from the intra-assay replicate determinations on quality control sera was 5.4 per cent at 4.7 ng/ml and 5.2 per cent at 11.4 ng/ml. The inter-assay variation calculated over a three week period on 65 assays was 8.7 per cent for 4.4 ng/ml and 5.9 per cent for 10.2 ng/ml. In order to determine accuracy of the assay the Nichols Institute Diagnostics Human Osteocalcin RIA was compared to the Nichols Institute Reference Laboratory Osteocalcin assay. A sample (N = 131) was assayed by each method. Least squares regression analysis was performed on the comparative data. A correlation coefficient (r) = 0.92 was obtained.

Statistical Methods

The SPSS package was used to complete the analysis of the data. Following collection of the pre test data, student t-tests were used in order to assess whether there were any

differences between the two groups with respect to anthropometry, bone mineral density, bone mineral content, cardiovascular fitness and bone metabolism, measured using the markers urinary hydroxyproline and serum osteocalcin. Once all data had been collected correlations were performed on the post test data between all variables. As there were poor relationships among the variables tested student t-tests were used to analyse each variable separately, and locate differences in the data. Stepwise multiple regression analysis was used to find which of the dependent variables of height, weight and the three cardiovascular fitness variables would best predict bone mineral density and bone mineral content at all sites measured.

In order to assess the relationship between cardiovascular fitness, and bone mineral density and content, the percentage difference in cumulative heart rate was used to divide the experimental group into two separate categories. Those above the mean change in cardiovascular fitness of 6.13 per cent and those below this mean. An ANOVA was performed with the control group as the third group.

CHAPTER 4

RESULTS

This section examines the pre and post test data of the subjects within the exercise and non-exercise groups following the 16 week exercise training program.

Pre test data

Examination of the pre test data for both control and experimental groups revealed no significant differences between the two experimental groups with respect to anthropometry (Table 4.I) and cardiovascular fitness (Table 4.III). Similarly, measures of bone composition (Table 4.IV) and bone metabolism (Table 4.VI) did not differ between the groups. Raw data for these results are presented in Appendix XV and tables containing student t-test for the pre test results are in Appendix XXIII.

There were no significant differences between the pre and post anthropometric data for exercise and non-exercise groups. This result reflected a high degree of variability in the data relating to weight . Following the exercise training program the exercise group lost an average of 2.21 per cent in weight (Figure 4.1.). In comparison, the non-exercise group gained an average 0.48 per cent in weight.

Table 4.I. Age and anthropometric data of the pre and post test results for exercise and non-exercise groups.

Group	Age	Height	Weight	
Exercise			Pre test	Post Test
Mean	22.6	178.4	74.59	72.94
SD	2.3	6.2	10.51	9.44
Control				
Mean	21.8	178.9	77.99	78.37
SD	1.9	4.4	6.04	7.20

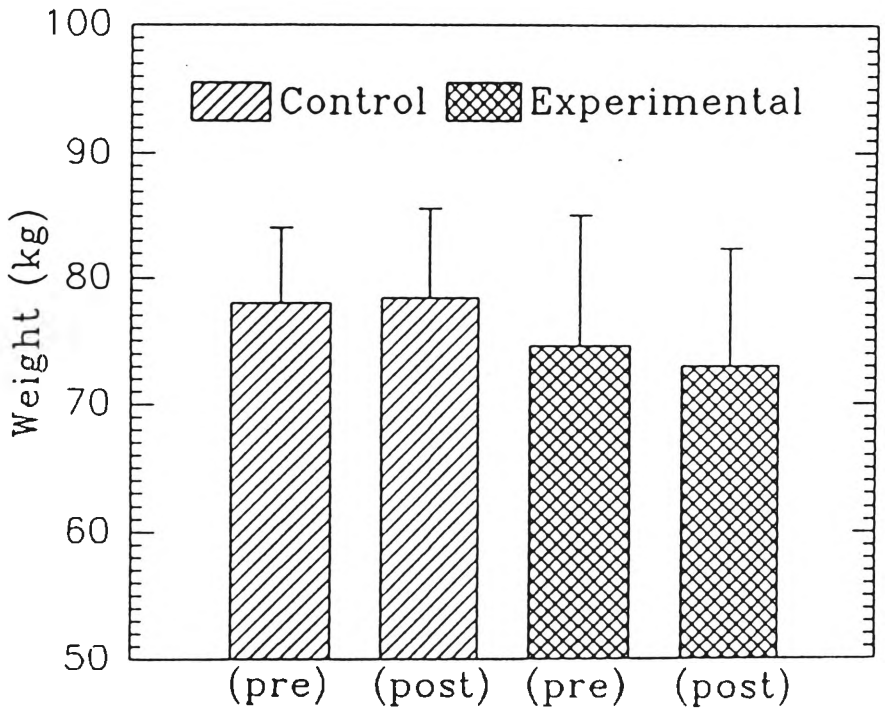


Figure 4.1. Differences in weight following the 16 week running program in the exercise and non-exercise groups (Mean ± SD).

Training History

Table 4.II provides means for the information recovered from the subjects' training diaries. On average, subjects attended 22 from a total of 48 runs, although 2 supervised runs were cancelled due to inclement weather. This amounted to an average of 40 minutes/week for each subject. To compensate for omissions in attendance, subjects were required to complete non-supervised make-up runs and maintain a record of the extent of these running sessions in an activity diary. Analysis of the diaries revealed that non-supervised runs amounted to an average of 28 minutes/week. By combining both supervised and unsupervised runs it was found that 1 hour and 8 minutes/week (71.8 per cent) of the running program was successfully completed by the exercise group. Total weekly running time throughout the 16 week program was on average just over 1.5 hours/week. The length of the runs varied over the 16 weeks, from approximately 5 kms/week in the first week to approximately 30 kms/week for the final 3 weeks of the running program. Raw data from the activity diaries is tabulated in Appendix XIII.

The experimental group was also asked to include in their diaries any additional activity undertaken over the 16 week intervention period. This amounted to a weekly average of 4.5 hours/week of additional weight-bearing activity for each subject. Control subjects were similarly required to record their activity which was an average of just over 1 hour/week, for the 16 week period. The additional activities were varied and included cycling, rollerblading, jogging, walking, soccer, gym, basketball and tennis. The amount of additional exercise varied considerably in the exercise group and ranged from zero to 10.5 hours/week. The range in the non-exercise group was from 18 minutes/week to 3 hours and 18 minutes/week.

Table 4.II. Mean scores from the information recovered from the participants activity diaries.

Group	No. supervised runs /46	Supervised runs (min)	Unsupervised runs (min)	Additional Ex. min/week
Exercise	22	649	461	272.4
Non-Ex.				74

Cardiovascular Fitness

The exercise group showed a significant ($P < 0.05$) improvement in cardiovascular fitness following the 16 week running program (Table 4.III). As can be seen in Figure 4.2. the cumulative heart rate (beats) of the exercise group showed a significant mean decrease between the pre and post test results ($P < 0.01$). The results from the first and final maximal exercise test revealed that the values for time to exhaustion (minutes) were also significant ($P < 0.001$) between the exercise and non-exercise group. This represented a mean increase in time on the treadmill from 10.5 to 11.8 minutes for the exercise group, whereas the time for the non-exercise group was reduced from 10.2 to 9.7 minutes.

Table 4.III. Mean and (SEM) values of pre and post test scores for measures of cardiovascular status.

Fitness Measure	Exercise Group		Non-exercise group		t value
	Pre test	Post test	Pre test	Post test	
Cumulative Heart Rate (beats)	1861.83 (66.21)	1754.83 (82.58)	1697.00 (90.12)	1698.40 (85.56)	2.94*
% change	-6.13		0.16		2.70*
Time to Exhaustion (min)	10.5 (0.5)	11.8 (0.3)	10.2 (0.5)	9.7 (0.5)	4.92*

* = significant ($P < 0.05$)

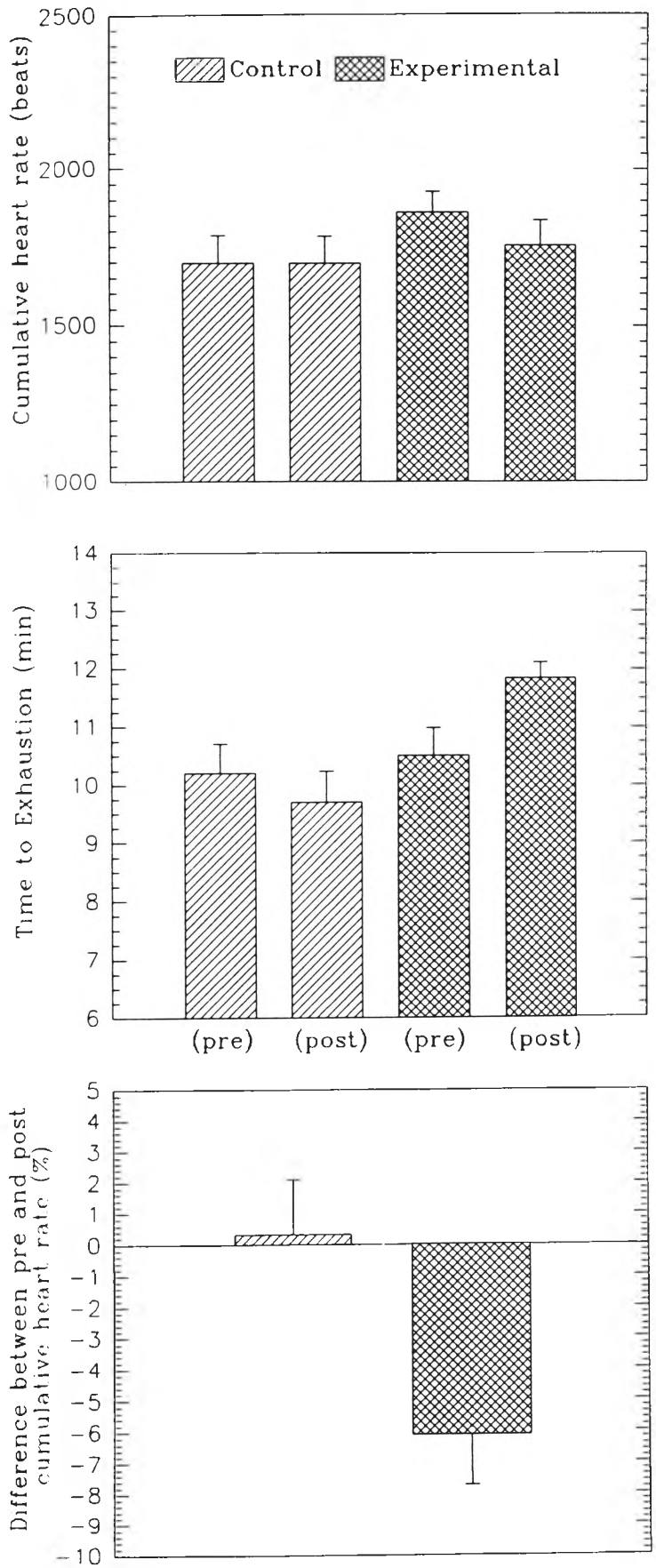


Figure 4.2. Pre and post exercise measures of cardiovascular fitness; cumulative heart rate (beats), time to exhaustion (min) and the difference between pre and post cumulative heart rates (%) (Mean ± SEM).

Bone Composition

Bone mineral density and bone mineral content were assessed at anatomical sites located on the proximal aspect of the femur and the lumbar vertebrae. Bone mineral density is a measure of the bone mineral mass divided by the bone area, whereas bone mineral content is the total mass of the bone mineral within the specified region of interest. The femoral measurements included the femoral neck, greater trochanter and Ward's triangle, while the lumbar vertebrae L2-L4 were measured individually and as an average of all three. No significant increase was found between exercise and non-exercise groups for BMD and BMC at any of the femoral sites, (Table 4.IV and Figure 4.3 and 4.4).

Similarly, no significant increase was found between the groups on these measures for BMD and BMC at L2, L3 and L2-L4. The pre and post test means (SEM) for BMD of the lumbar spine are presented in Figure 4.5 and for BMC in Figure 4.6 and the raw data in Appendix XV-XVIII. However as can be seen in Table 4.IV a significant decrease between the exercise and non-exercise groups was found for BMD ($P < 0.05$) and BMC ($P < 0.05$) at the 4th lumbar vertebrae.

There was individual variability in the bone mineral density of the exercising subjects following the intervention period, however variability was not significant. Measures ranged from 0.805 $g.cm^{-2}$ to 1.359 $g.cm^{-2}$ for the femoral neck and from 0.880 $g.cm^{-2}$ to 1.398 $g.cm^{-2}$ for the 2nd to 4th lumbar vertebrae. One subject (R7) began the experiment with a bone mineral density of 0.700 $g.cm^{-2}$ at the femoral neck. Following the intervention period bone mineral density at this site increased to 0.805 $g.cm^{-2}$, an increase of 15 per cent. A graphic representation of this result versus the mean of the group for this site is presented in Figure 4.7.

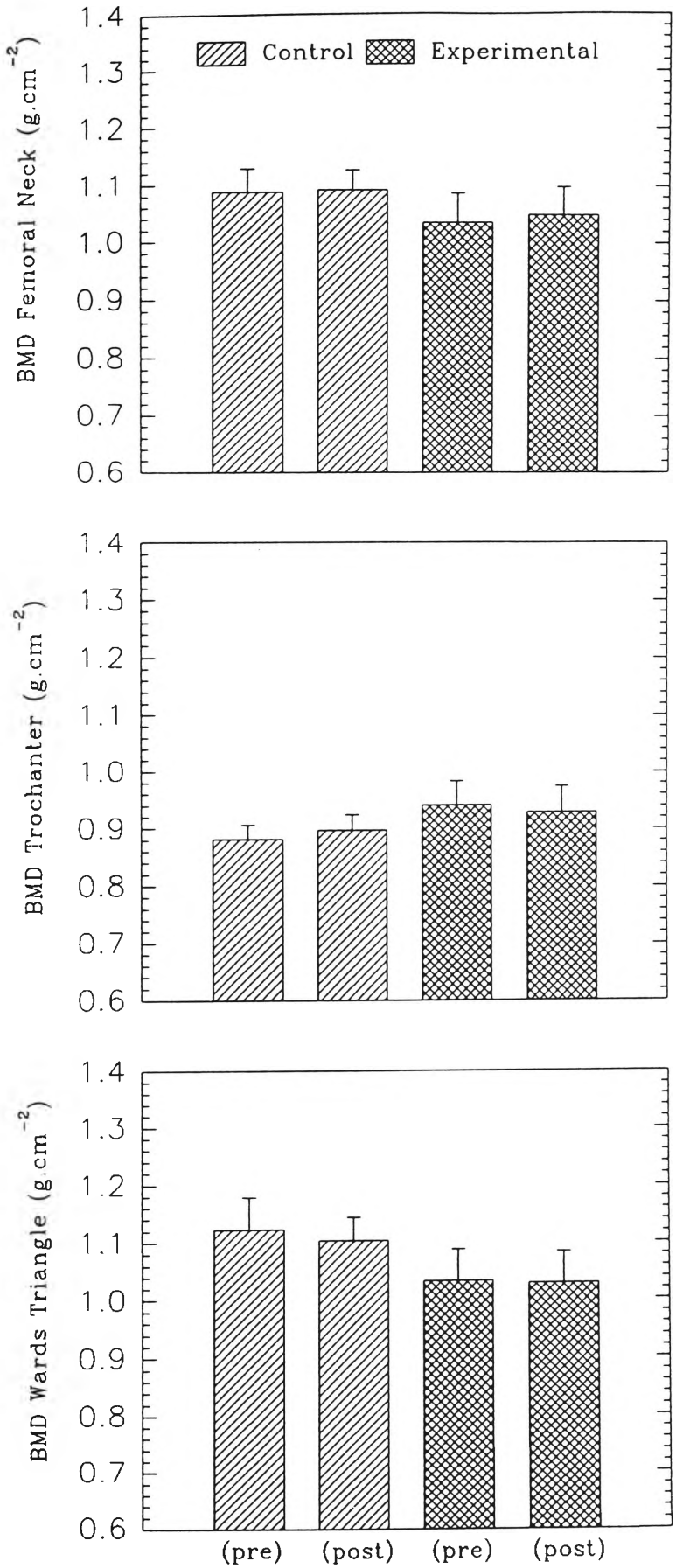


Figure 4.3. Pre and post exercise measures for bone mineral density (g.cm-2) at the femoral neck, greater trochanter and Ward's triangle in the exercise and non-exercise groups (Mean ± SEM).

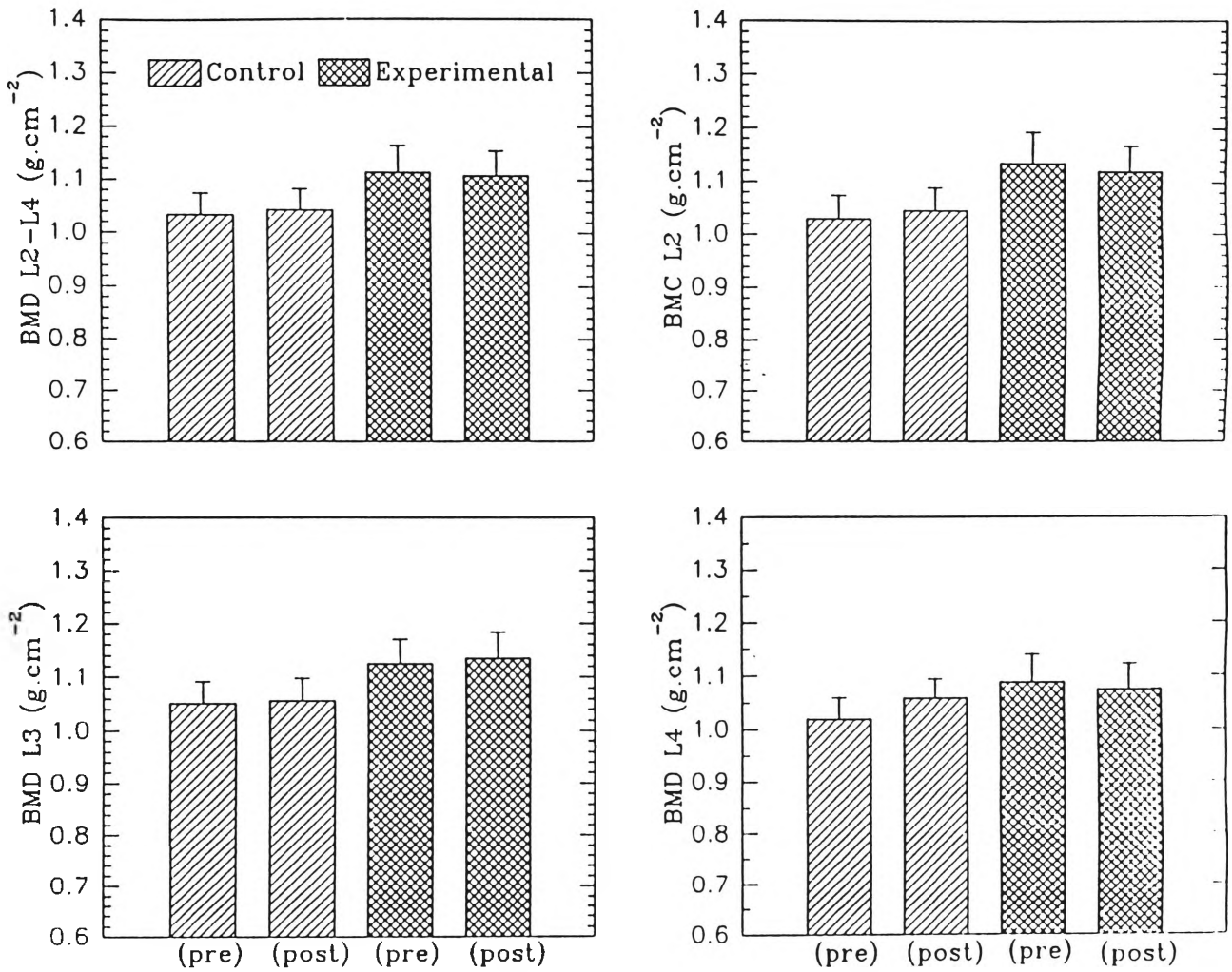


Figure 4.4. Pre and post exercise measures of bone mineral density ($g.cm^{-2}$) from L2-L4, and the individual vertebrae L2, L3, L4 of the exercise and non-exercise groups (Mean $\pm$ SEM).

Table 4.IV. Mean and (SEM) values for pre and post test bone mineral density ($g.cm^{-2}$) and bone mineral content ($g.cm^{-1}$) at all sites.

Site Measured BMD	Exercise Group Pre test	Exercise Group Post test	Non-exercise group Pre test	Non-exercise group Post test	t value
Femoral neck	1.035 (0.051)	1.048 (0.050)	1.088 (0.040)	1.092 (0.035)	0.49
Greater Trochanter	0.941 (0.043)	0.929 (0.045)	0.882 (0.025)	0.898 (0.026)	1.33
Ward's Triangle	1.033 (0.050)	1.029 (0.055)	1.123 (0.055)	1.103 0.040)	0.62
L2-L4	1.114 (0.050)	1.108 (0.047)	1.033 (0.041)	1.043 (0.040)	1.12
L2	1.135 (0.059)	1.121 (0.047)	1.030 (0.044)	1.046 (0.042)	1.12
L3	1.125 (0.045)	1.125 (0.049)	1.052 (0.040)	1.057 (0.041)	0.20
L4	1.089 (0.051)	1.074 (0.049)	1.020 (0.040)	1.059 (0.035)	2.41*
BMC					
Femoral neck	3.97 (0.26)	3.93 (0.29)	4.27 (0.15)	4.20 (0.12)	0.25
Greater Trochanter	13.40 (0.90)	12.20 (1.33)	12.59 (0.52)	11.76 (0.92)	0.27
Ward's Triangle	1.05 (0.06)	1.03 (0.06)	1.08 (0.05)	1.10 (0.04)	1.32
L2-L4	50.00 (2.81)	49.15 (2.43)	48.19 (2.20)	48.41 (2.07)	1.30
L2	16.04 (1.09)	15.75 (0.89)	14.87 (0.70)	14.91 (0.65)	0.86
L3	16.52 (0.87)	16.67 (0.90)	16.37 (0.69)	16.35 (0.65)	0.39
L4	17.44 (0.90)	16.80 (0.76)	16.95 (0.88)	17.26 (0.84)	2.34*

* = significant ($P < 0.05$).

Post-test correlations revealed significant ($P < 0.05$) associations between the BMD and BMC measures at the different sites, with the exception of the L2-L4 BMD and L3 BMC which were not correlated with the Ward's triangle area. These correlation coefficients are presented in Appendix XXVI. As there were no differences in anthropometry between the exercise and non-exercise group their results were pooled, in order to assess associations with BMD/BMC. Table 4.V contains the correlation coefficients for height and weight. Height showed significant ($P < 0.05$) associations with BMD of the femoral neck ($r = 0.5895$), Ward's triangle ($r = 0.6541$) and the 3rd lumbar vertebra ($r = 0.4301$), whereas weight was only correlated with BMD of the Ward's triangle region ($r = 0.5229$). In contrast, with the exception of the greater trochanter, all BMC sites were significantly ($P < 0.05$) associated with height. Weight was significantly ($P < 0.05$) correlated with BMC of the femoral neck ($r = 0.5953$), Ward's triangle ($r = 0.5263$), and L2-L4 ($r = 0.4307$).

Table 4.V. Correlation coefficients of the anthropometric variables and bone mineral density and bone mineral content.

BMD	Femoral Neck	Greater Trochan.	Ward's Triangle	L2-L4	L2	L3	L4
Weight	0.3225	0.0613	0.5229*	0.2229	0.1876	0.2642	0.1027
Height	0.5895*	0.3214	0.6541*	0.3695	0.3112	0.4301*	0.2917

* = significant correlation ($P < 0.05$)

BMC	Femoral Neck	Greater Trochan.	Ward's Triangle	L2-L4	L2	L3	L4
Weight	0.5953*	0.0892	0.5263*	0.4307*	0.4060	0.4177	0.3845
Height	0.7302*	0.4102	0.6546*	0.6274*	0.5650*	0.6290*	0.5711*

* = significant correlation ($P < 0.05$)

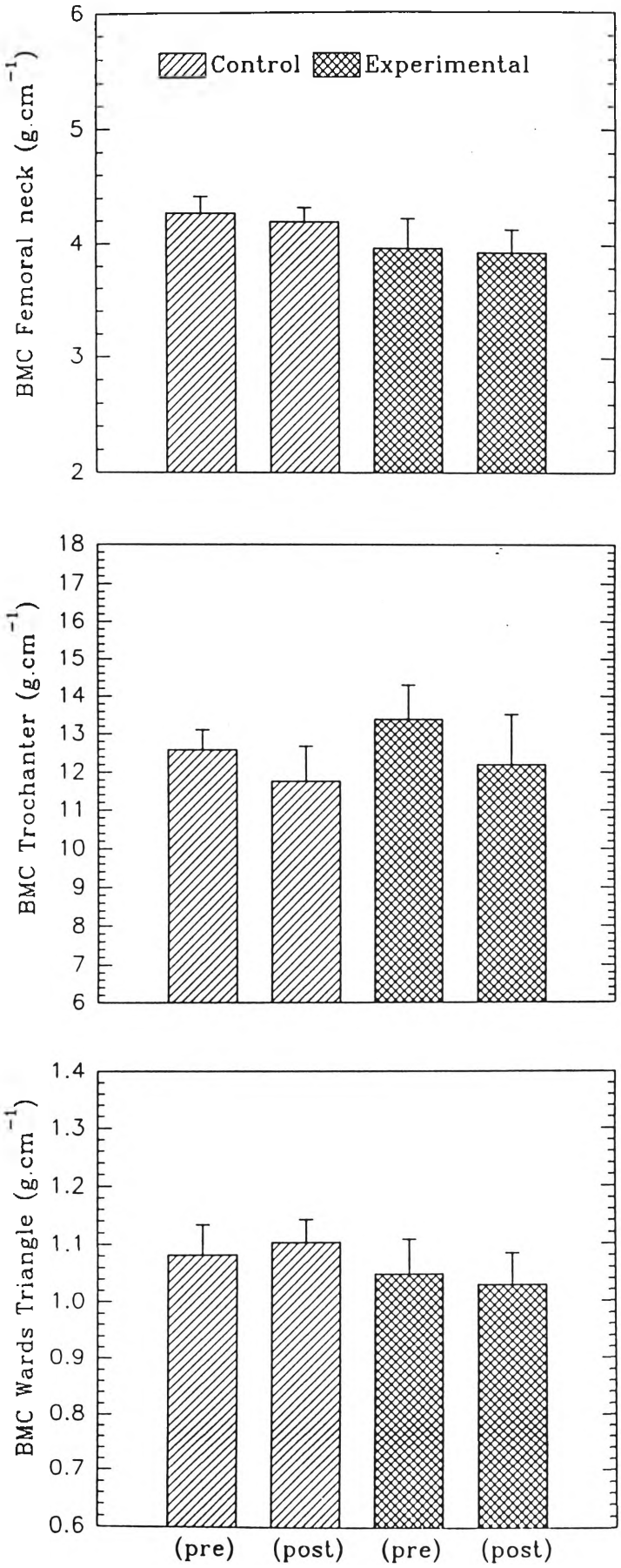


Figure 4.5. Pre and post exercise measures of bone mineral content ($g.cm^{-1}$) at the femoral neck, greater trochanter and Ward's triangle in the exercise and non-exercise groups (Mean ± SEM).

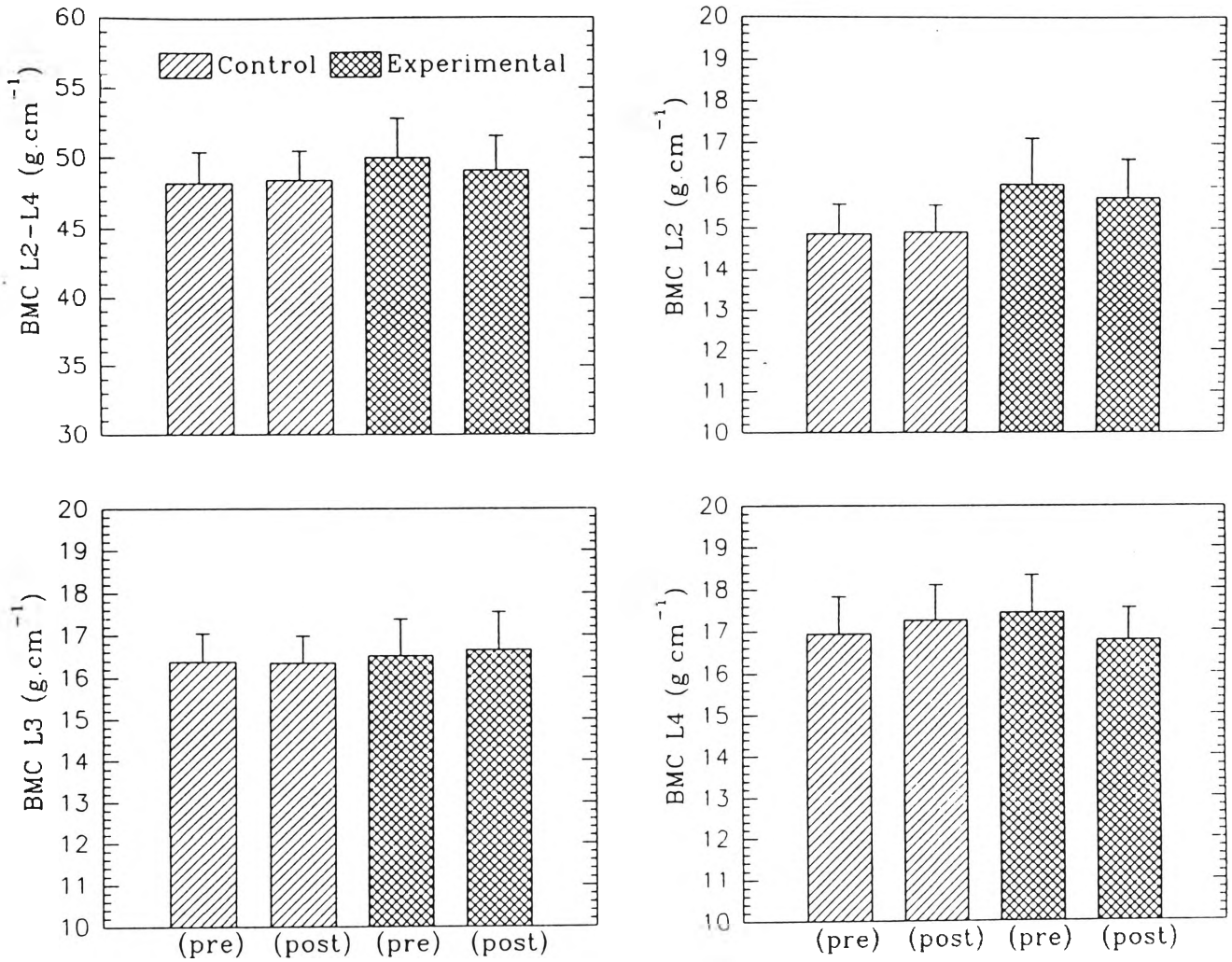


Figure 4.6. Pre and post exercise measures of bone mineral content (g.cm-1) from L2-L4, and the individual vertebrae L2, L3, L4 of the exercise and non-exercise groups (Mean ± SEM).

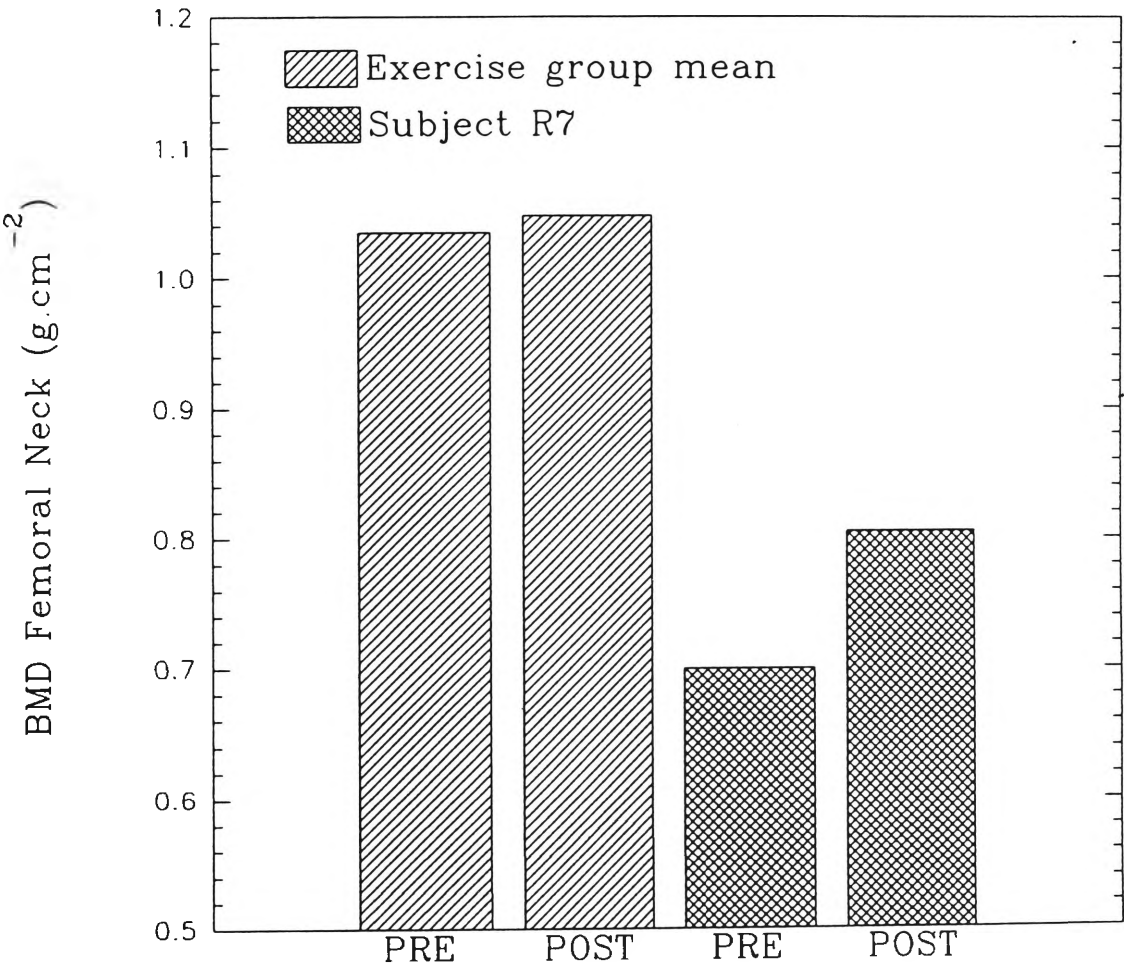


Figure 4.7. Pre and post exercise measures of bone mineral density (g.cm^{-2}) at the femoral neck for subject R7 versus the pre and post test mean of the exercising subjects at this site.

Biochemical Analysis

No significant increase was found in pre and post test scores between the exercise and non-exercise groups for the bone markers serum osteocalcin and urinary hydroxyproline, indicators of bone formation and resorption respectively, (Figure 4.7 and Appendix XIX). A trend toward a decrease was evident between the pre and post data for both the exercise and non-exercise groups in their levels of serum osteocalcin and urinary hydroxyproline (Table 4.VI). Over the 16 week exercise period serum osteocalcin concentration decreased by an average of 14.2 per cent and urinary hydroxyproline by 17.9 per cent in the non-exercise group. By contrast, members of the exercise group increased serum osteocalcin and urinary hydroxyproline concentration by an average of 2.5 per cent and 7.6 per cent respectively. Normal variability in the data was evident for both biochemical analyses, however the variability was not significant.

Table 4.VI. Mean (SEM) values of pre and post test scores for the biochemical bone markers urinary hydroxyproline (mol/mmol creatinine) and serum osteocalcin (ng.ml $^{-1}$).

Biochemical bone marker	**Exercise group**		**Non-exercise group**		**t value**
	Pre test	**Post test**	**Pre test**	**Post test**	
Serum Osteocalcin	12.1 (1.6)	12.4 (1.1)	15.5 (2.6)	13.3 (1.4)	0.69
Urinary Hydroxyproline	17.2 (2.5)	18.5 (1.9)	20.1 (1.9)	16.5 (1.3)	1.48

Post test correlation coefficients between bone mineral density and content and serum osteocalcin and urinary hydroxyproline are shown in Appendix XXVI. This analysis resulted in a significant ($P < 0.05$) negative association between serum osteocalcin and bone mineral content of the 3rd lumbar vertebrae ($r = -0.5206$). No other significant correlations were found between serum osteocalcin or urinary hydroxyproline and BMD/BMC at any of the sites measured.

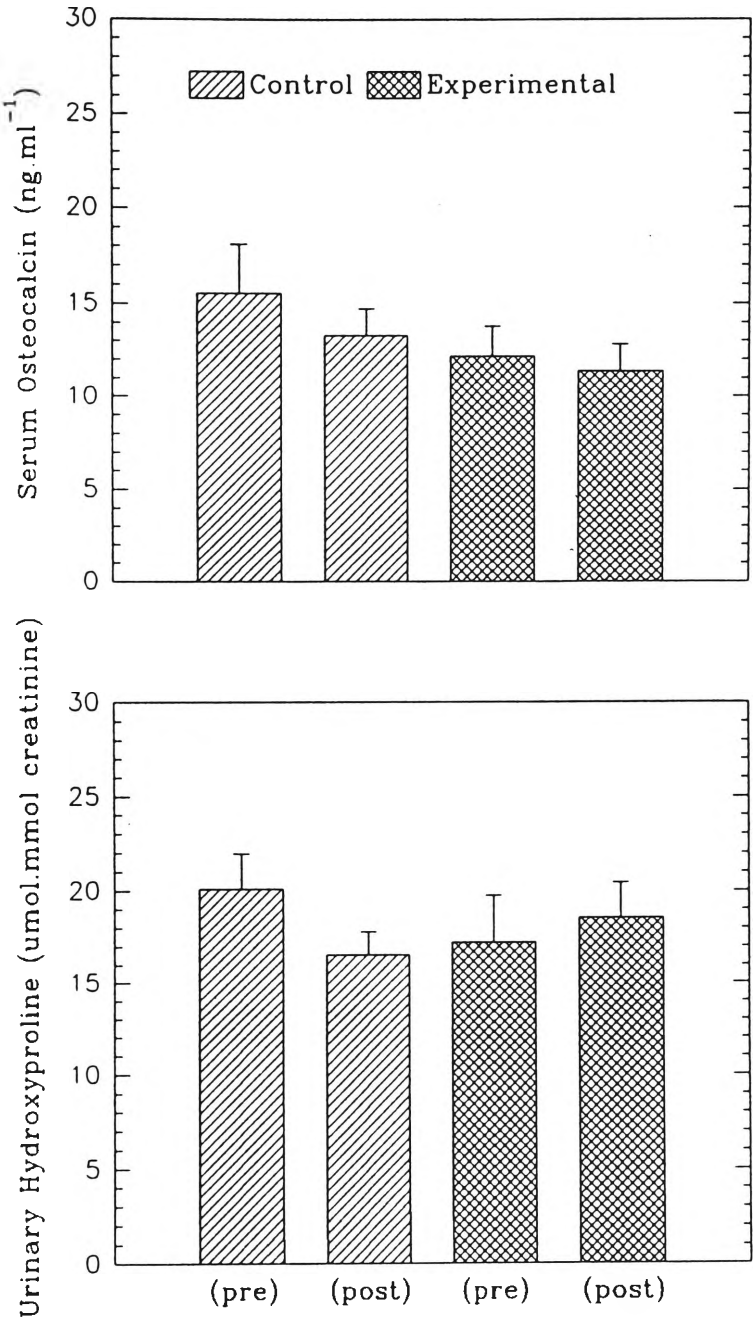


Figure 4.8. Pre and post exercise levels of serum osteocalcin (ng.ml^{-1}) and urinary hydroxyproline (umol.mmol creatinine) in the exercise and non-exercise groups (Mean ± SEM).

Fitness and Bone Density

As previous studies had demonstrated a relationship between fitness and bone mineral density the data was further analysed to test this relationship. As can be seen in Table 4.IV no significant differences were found between exercise and non-exercise groups for BMD and BMC at the proximal femur and L2-L4, L2 and L3 following the running program. Therefore it was thought that a change in BMD and BMC may be present in those subjects that had the greatest change in fitness. The cardiovascular fitness data was used to categorise the exercise subjects into two separate groups according to those subjects that recorded a decrease in cumulative heart rate above the mean of -6.13 per cent and those below the mean. This procedure revealed no significant differences for those subjects who experienced the greatest change in cardiovascular fitness. Statistical reports for this procedure are presented in Appendix XXV.

All correlation coefficients for cardiovascular fitness are presented in Appendix XXVI. There was no association between the decrease in cumulative heart rate and BMD/BMC at any of the sites measured. This was also the case for the other indicator of cardiovascular fitness, time to exhaustion.

A stepwise multiple regression procedure was used to assess the independent contributions of the post-test fitness data, weight and height to BMD and BMC at all sites measured. Height was the single most predictive factor as the fitness data, weight failed to reach significance. Height accounted for 35 per cent of the variation in BMD of the femoral neck ($P < 0.01$), 43 per cent of the variation in BMD of Ward's triangle ($P < 0.001$) and 18 per cent of the variation in the 3rd lumbar vertebrae ($P < 0.05$). Height was also the single most predictive factor, accounting for 53 per cent of the variation in BMC of the femoral neck ($P < 0.001$), 43 per cent in Ward's triangle ($P < 0.001$) and 39 per cent in the average of the three lumbar vertebrae ($P < 0.01$). Each individual vertebrae was also significant with height predicting 32 per cent of the variation in the 2nd lumbar vertebrae ($P < 0.01$), 40 per cent in the 3rd lumbar vertebrae ($P < 0.01$) and 33

per cent in the 4th lumbar vertebrae ($P < 0.01$). Statistical reports are presented in Appendix XXVII.

CHAPTER 5
DISCUSSION

Numerous cross-sectional studies (Nilsson and Westlin, 1971; Jones et al. 1977; Block et al. 1986; Block et al. 1989; Orwoll et al. 1989; Pocock et al. 1989; Bevier et al. 1989) have shown those engaging in an active lifestyle such as athletes, have a greater BMD and BMC than sedentary individuals. The amount of bone mineral attained at skeletal maturity is considered to be an individuals' peak bone mass, and exercise may be an important contributor to maximising this parameter. An elevated peak bone mass may have a protective function against the loss of bone and related consequences, which commonly occur with aging. Positive benefits with respect to exercise and bone density have been shown in children, and those with low bone density such as the elderly. Longitudinal training studies have been focused on the acquisition of bone mass and density in female populations and there is a dearth of information derived from longitudinal investigations of changes in the BMD of young adults, and especially a young male population.

The aim of the current investigation was to evaluate the effects of a 16 week running training program on bone composition and bone metabolism in young sedentary males with an average age of 22.6 years. It was hypothesised that bone mineral density and bone mineral content measured at sites on the proximal femur and vertebrae of the lumbar spine, together with biochemical estimates of bone metabolism would increase following the exercise program.

There were no differences between the exercise and control group for the values of BMD and BMC at the majority of skeletal sites following the exercise program. A decrease in BMD and BMC of the 4th lumbar vertebrae was the only change. The values obtained for BMD and BMC were within the range of data derived from other research (Rico et al. 1992; Bonjour et al. 1991; Geusens et al. 1991) involving males of similar age.

The lack of a change in BMD and BMC following the exercise program was consistent with earlier investigations (Dalen and Olsson, 1974; Cavanaugh et al. 1988; Nelson et al. 1991). In these studies exercise intervention ranged from 3-12 months, and the populations consisted of either males that were on average 5 years older than subjects in this study, or premenopausal and postmenopausal women respectively. In contrast, MacDougall et al. (1992) found male runners aged 20-45 years had a higher BMD of the tibia compared to sedentary controls. Their subjects had a 2 year history of running at an intensity and mileage of 15-20 miles/week which was similar to that used in this investigation. Neither running volume or intensity in the latter study affected BMD of the lumbar spine which may suggest that running affects the density of each site independently.

The disparity in the results of the different exercise studies may reflect differences in the nature, duration and intensity of the exercise program. Earlier research has shown that changes in the structure and composition of bone may be specific to the intensity of the activity (Margulies et al. 1986; MacDougall et al. 1992). The program used in the present investigation averaged 1.5 hours of running per week and was of moderate intensity, as determined from a percentage of each subject's maximal heart rate. In contrast to the results of the present investigation, increases of up to 11 per cent in BMD and BMC have been shown in the tibia of young males following extremely intensive exercise (Margulies et al. 1986; Leichter et al. 1989). Intensity was characterised by activity lasting 8 hours a day, 6 days a week, for 14 weeks and with a load for some subjects which was sufficient to produce injury and stress fracture. These results suggest that bone is sensitive to a high intensity exercise stimulus reflecting structural adaptations in bone tissue. MacDougall and associates (1992) showed that as running mileage and intensity was increased above 64 miles/week, BMD of the tibia decreased with a corresponding increase in cross-sectional area at this site. Similar changes in geometry were found in the long bones of dogs subjected to high repetitive stress (Chamay and Tschantz, 1972). In contrast to the cortical bone of the tibia, the bone sites examined in this study contained

a majority of trabecular bone. Although not measured in this study, it is unlikely that the geometry of the proximal femur and lumbar spine were modified as they were not exposed to high intensity repetitive exercise.

The precise level of loading induced by physical activity which is required to optimise the genetic potential for bone density is poorly defined, however it is likely that threshold levels exist beyond which the acquisition or resorption of bone occurs. Frost (1993) proposed that strains above a certain threshold may initiate modelling and changes in the tissue's architecture, such as an increase in cross-sectional area which in turn may reduce strains. Conversely, strains below a certain threshold may initiate bone resorption. Rubin and Lanyon (1984) using an avian ulna model have shown the existence of a peak strain magnitude threshold. One hundred load cycles per day with peak strains of 500 microstrain were insufficient to prevent a decrease in the cross-sectional area of the ulnae. However, when peak strain was increased to 1000 microstrain, bone area was maintained and strains above this level were associated with new bone formation. However, such experiments are not representative of an exercise stimulus and extrapolation to intervention studies involving human subjects is difficult. The results of the present investigation would suggest that the magnitude of the exercise program did not reach the threshold to stimulate new bone formation, but was sufficient to maintain BMD and content within normal levels.

The duration of an exercise program is an important consideration when designing exercise programs to initiate skeletal adaptation. Cross-sectional studies have shown that athletes particularly have a larger bone mass compared to sedentary populations, perhaps as a result of their involvement in physical activity for a number of years (Nilsson and Westlin, 1974; Block et al. 1986; Block et al. 1989; Orwoll et al. 1989). Longitudinal analyses have shown that 9 months of running training at a moderate intensity in older men (Williams et al. 1984) and postmenopausal populations (Dalsky et al. 1988; Rundgren et al. 1984) was successful in increasing BMD and content. The present study

was concerned with assessing the benefits of a short-term exercise program of moderate intensity, as previous short-term training studies have only considered intensive activity (Margulies et al. 1986; Leichter et al. 1989).

In contrast to previous studies (Dalen and Olsson, 1974; Margulies et al. 1986; Leichter et al. 1989) an attempt was made to determine the total activity levels of exercise and control subjects over the 16 week period. Previous training studies have not included records of informal exercise, thereby limiting interpretation of the results of these studies as the total activity cannot be determined and may be underestimated. Therefore activity diaries in the present investigation provided a reliable means of assessing the daily training histories for both formal and informal activity of both exercising and control subjects. Informal activity undertaken by the exercise group approximated 4.5 hours/week and included walking, bushwalking, martial arts, and cycling, whereas the control group participated in approximately 1.2 hours/week of informal activity. Total activity for the exercise group was increased to approximately 6 hours/week when the running program and informal activity were combined. The strains produced by the additional aerobic activity may have been of insufficient magnitude to influence BMD and content at the local tissue level but the effect on the cardiovascular system was sufficient to show a positive adaptation. This reflects the response of different systems to exercise, whereby bone functional mass may be determined by the balance between systemic mechanical and physiological influences at the local tissue level.

The total activity for exercise and control subjects ranged from 6 hours/week to 1.2 hours/week respectively. Within this activity range there was no difference in BMD and BMC between the exercise and control groups over the 16 week period. The control group in this study were not true controls as they participated in some activity. For a true control group subjects would need to be immobilised, which is obviously not possible. Thus there seems to be a broad range of mechanical loading between the threshold levels for apposition and resorption. Therefore maintenance of BMD and BMC may be

achieved with a low level of activity in young male subjects. This result is in contrast to a detailed physical activity survey of pre and postmenopausal women (Cheng et al. 1990). They showed that women who participated in over 9 hours/week of activity had a greater BMD than those who participated in less than 3 hours/week of activity. Those women who took part in between 4-8 hours/week of activity also had a greater BMD than the 3 hour/week group. The age of subjects and density measures prior to involvement in exercise may be a factor in determining whether physical activity will influence BMD as evidenced by the above study.

The finding of a decrease in BMD and BMC at the 4th lumbar vertebrae was surprising as it could not be supported by other longitudinal training studies. Lower vertebral bone density has been reported in male long distance runners compared to a non-running control group (Bilanin et al. 1989). The authors of this study could offer no explanation for this result.

One possible explanation for the decrease at the 4th lumbar vertebrae arises from the remodelling space theory formulated by Jaworski (1976). The remodelling space is the total volume of all bone which is temporarily missing as a result of the delay between resorption and formation in the normal steady state remodelling environment. This may account for a decrease of approximately 5 per cent of trabecular BMD. Following the formation phase the newly deposited primary mineralised bone has a low density. The period of time for one remodelling cycle is referred to as sigma, and in cortical bone takes approximately 3-4 months and in trabecular bone approximately 2-3 months (Parfitt, 1980). Biochemical markers of bone resorption and formation in this investigation revealed that bone metabolism remained unaltered following the running program. One may only speculate at this point that the second density measurement following the running program may have coincided with primary mineralisation or the resorption phase of remodelling when bone was temporarily missing. However this does not explain why the effect was localised at the 4th lumbar vertebrae.

The difference in responses at the 4th lumbar vertebrae and proximal femur and other vertebrae studied suggests that the exercise effect may be site specific. Ormerod and associates (1990) showed that an increase in running mileage from 5 miles/week to 40 miles/week had a positive effect on bone density of the lower leg and dominant thigh but did not effect changes in the lumbar vertebrae. The results of animal research has shown that geometric, composition and growth properties may vary in different bones presumably as a function of the different loading histories at a particular site. Li et al. (1991) examined the effect of strenuous exercise on the immature bone of rats. They found that the cross-sectional area of two sites differed, with an increase at the second metatarsus and a decrease in the tibia. Furthermore, Forwood and Parker (1991) showed significantly greater appositional growth at the endocortical surface of the mid-diaphysis of the rat femora compared to a significantly reduced appositional growth in the endosteal surface of the tibia, following approximately 20,000 loading cycles of treadmill running per day. Differing strain environments in bones may determine the site specific responses as a result of mechanical loading (Lanyon et al. 1975).

The exercise program was of sufficient intensity to effect an increase in cardiovascular fitness of the exercising subjects. This increase was evidenced by a decrease in cumulative heart rate and a 12 per cent improvement in their time to exhaustion on the treadmill, whereas the non-exercise group experienced a 5 per cent decrease in this measure following the 16 week program. This result is in accordance with previous studies (Norris et al. 1990; Swaine et al. 1992; Somers et al. 1991) that reported a change in fitness following 10, 16 and 24 weeks of exercise, using a frequency and intensity similar to that of the current experiment.

No association was found between the changes in cardiovascular fitness and bone mineral density or bone mineral content. This result was supported by previous studies that have also assessed fitness using a maximal exercise test to exhaustion (Bevier et al. 1989; Dalsky et al. 1988). In contrast, some earlier research (Chow et al. 1986, 1987; Pocock

et al. 1986, 1989) has shown an association between fitness and BMD and content. Several factors may account for this difference including the differences in age of the subjects and the methods of fitness assessment. These previously cited studies used indirect methods to assess fitness in postmenopausal women that involved steady-state observations during bicycle ergometry, while subjects in the present investigation exercised on a treadmill with progressive increments until exhaustion. Prediction of fitness is associated with a 10-15 per cent error (Davies, 1968; Roweel et al. 1964; Taylor et al. 1963) and this inaccuracy may have contributed to their favourable correlations. The authors concluded that the positive association between fitness and BMD may be due to both factors sharing a common physiological basis rather than being causally related.

The mechanical stimuli that aerobic activity itself provides the skeleton and the physiological response of body systems to physical activity may both contribute to the relationship between cardiovascular fitness and BMD and content. The lowering of normal circulating testosterone levels is one physiological response known to occur during intensive physical activity (Hackney et al. 1988; Wheeler et al. 1984). The lowering of testosterone has also been associated with low BMD (Bilanin et al. 1989; Ormerod et al. 1990). This may not be the case in this investigation as exercise was conducted at a moderate intensity. Acute and chronic moderate intensity exercise is known to increase secretion of both growth hormone and insulin-like growth factor-I (IGF-I) (Kraemer et al. 1992; Felsing et al. 1992). Insulin-like growth factor-I mediates the anabolic effect of growth hormone, and IGF-I has been shown to have a positive relationship with BMD of the femoral neck, lumbar spine and distal radius (Kelly et al. 1990). The long term response of hormones to exercise may be determined by the intensity and duration of the training program (Felsing et al. 1992). As hormone levels were untested in this study one does not know whether the mechanical and physiological stimulus created by the exercise program was sufficient to influence hormone levels and BMD and content in exercising subjects.

The measurement of serum osteocalcin and urinary hydroxyproline provided an assessment of bone formation and bone resorption respectively. No alterations in either serum osteocalcin or urinary hydroxyproline levels were found following the exercise program. In addition there was no evidence of any association between the bone markers and BMD or BMC in the present study. The mean values for both groups were within clinical ranges (Hypronostican manual, 1989; Nichols Institute Diagnostics, 1992) and showed a close relationship with values reported in previous studies that did not involve an exercise intervention (Kelly et al. 1989; Cole et al. 1985). No human studies were found concerning the effect of longitudinal training programs on osteocalcin and hydroxyproline by which to provide a direct comparison with the results found in this investigation. Only one cross-sectional study that examined women of differing ages found no association between osteocalcin concentration and BMD of the femoral neck and lumbar spine (Kelly et al. 1990). The authors offered no explanation for this result, however the considerable variation in BMD over the ages investigated (19-83 years) may have led to the non-significant result. The mean levels of both urinary hydroxyproline and serum osteocalcin in the exercise group remained stable over the 16 week period, with normal biological variation between subjects.

There was a trend towards a decrease for both urinary hydroxyproline and serum osteocalcin in the non-exercise group. This was interesting as it may represent less metabolic activity of the skeletal sites studied, and is indicative of the relative inactivity of the subjects in this group over the 16 week period. Evaluation of the activity diaries revealed that control subjects participated in as little as 1.2 hours of exercise per week, an amount that did not rise above the entry criteria for participation in this study.

When data for the exercising and control subjects were pooled, a relationship was found between weight and BMD/BMC and between height and BMD/BMC (Table 4.V). A stepwise multiple regression procedure using the post test data found height had the strongest predictive capacity, accounting for 18-43 per cent of the variability in bone

mineral density and 32-53 per cent of the variability in bone mineral content. Correlations have been previously reported between height, weight and BMD in children and adolescent subjects (Kroger et al. 1992; Miller et al. 1991; Picard et al. 1988) but not in subjects over 20 years of age. The age of epiphyseal plate closure is considered to be the threshold for correlations of anthropometric variables, as bone growth has stabilised and peak bone mass has generally been reached. Individual variation for the attainment of peak bone mass may vary within the third decade of life, therefore within this decade correlations between height, weight and BMD and BMC may need to be considered.

The age of subjects in the current investigation may have been an important factor in the lack of a change in BMD and BMC following the running program. During the period from conception to maturity bone mineral is acquired at a rapid rate and may reach a peak in the third decade of life (Geusens et al. 1986; Gotfredsen et al. 1987; Rico et al. 1992). Growth studies (Bonjour et al. 1991; Gilsanz et al. 1988) have provided evidence of the acceleration in skeletal growth during adolescence through to approximately 18 years of age when skeletal maturity is reached. The amount of bone mineral acquired during this time represents an interaction between the genotype, and environmental and lifestyle factors, in particular diet and physical activity. There is the possibility that members of the exercise group in the current investigation may have reached their peak bone mass (average age 22.6 years) prior to the exercise program. This may have limited their potential for an increase in BMD. Increases in bone density reported in previous investigations (Margulies et al. 1986; Leichter et al. 1989) were said to be associated with possible continued growth, as subjects were of relatively young age (18-21 years). The previous studies were limited as they did not include a control group to ascertain the possibility of increased mineralisation due to growth. As young bones still have the capacity to grow and peak bone mass has not yet been attained there may be an increased sensitivity to exercise and a greater capacity to increase bone mass and density.

In this investigation there was considerable individual variation in BMD and BMC which was expected as it reflects individual patterns of skeletal maturation. For example, density of the femoral neck ranged from a low 0.805 $g.cm^{-2}$ to a high of 1.359 $g.cm^{-2}$ following the exercise program. Closer examination of individual density patterns revealed an interesting case study that required closer investigation. One subject (R7) who began this study with a clinically low (Norland Western European standard) BMD of 0.700 $g.cm^{-2}$ in the femoral neck, increased BMD at this site by 15 per cent to 0.805 $g.cm^{-2}$ following the 16 week running program. In comparison, the mean change in the femoral neck for all subjects was 1.2 per cent (Figure 4.9). Increases were also evident in this subject at the greater trochanter and Ward's triangle, with both sites below 0.800 $g.cm^{-2}$ prior to the running program. A level of 0.700 $g.cm^{-2}$ is equivalent to the physiological level of a child 10-12 years of age (Bonjour et al. 1991). Individuals with a BMD between 0.850 $g.cm^{-2}$ and 1.000 $g.cm^{-2}$ are considered to be at an intermediate risk of bone injury (Riggs et al. 1982) and those with a BMD below 0.850 $g.cm^{-2}$ are at greater risk (Melton et al. 1986). Subject R7 was the oldest (27.1 years) member of the exercising group and he would have completed longitudinal growth at this age. He was 10 cm shorter than the average height measure, 2 kg lighter than the mean weight for the group pre test, and obtained the average weight measure post-test. This subject did not participate in any additional exercise that would influence this result. Bone mineral density of the remaining subjects was above 0.800 $g.cm^{-2}$, and showed no increase which may indicate the existence of a density threshold which when reached, could not be modified by short term moderate intensity exercise. This subject was also found to have a parallel increase in the markers of bone formation and resorption. Serum osteocalcin increased from 8.5 ng/ml to 10.6 ng/ml (24 per cent) and urinary hydroxyproline increased from 8.6 mmol/mol creatinine to 12.1 mmol/mol creatinine (40 per cent). This represents an increase in the metabolic activity of this subject's bone tissue as a result of the exercise program, which supports an effective increase in bone density.

The tenuous result found in the one subject may relate to the variability one may expect in young adults. Genotype may determine the limit of an individuals' skeletal response to environmental and lifestyle influences such as diet and physical activity which are important to the attainment of peak bone mass. Adolescence is characterised by accelerated mineralisation of the skeleton although the patterns of mineralisation vary according to an individual's genetic timetable and the manipulation of extrinsic factors such as diet and physical activity. Growth studies (Miller et al. 1991; Geusens et al. 1986, 1991) have shown density levels from age 5-12 years approximate 0.721 $g.cm^{-2}$ for the lumbar spine and 0.755 $g.cm^{-2}$ for the femoral neck, increasing to 0.910 $g.cm^{-2}$ and 1.171 $g.cm^{-2}$ respectively at maturity. Although there was no difference in the mean values between groups at the beginning of the current experiment this one individual was found to have an immature density for his age and the introduction of the exercise intervention elevated his low density close to a normal density value for his age at this site. It is possible that density levels on entry to the exercise program may be a critical factor in the adaptation or response of bone to an exercise stimulus. Identification of individuals with a lowered bone density prior to an exercise program may characterise subjects with the greatest potential for increasing bone mineral and also with the greatest risk of injury.

The individual case study result must be treated with caution as it was not a majority finding, however other evidence suggests that exercise intervention in skeletally immature subjects may increase bone mass and density. A hypothesis recently advanced by Eisman et al. (1993) suggests that exercise may have its greatest effect on bone density during skeletal development, when bone density is low. This hypothesis was based on studies which examined retrospectively the historical involvement in physical activity of children (Slemenda et al. 1991) and adults during their growth years (Kriska et al. 1988). Further support is provided by a study that reported BMD of osteoporotic women was equivalent to adolescent levels, and was successfully increased upon using an exercise intervention (Simkin et al. 1987). From this evidence and that of the case study it may be possible to

speculate that exercise during the adolescent growth period may advance bone mass above normal adolescent levels, allowing a substantial increase in bone mass before maturity is reached. The intensity and duration required to increase bone density in adolescents has not been thoroughly examined. It is known that intensive activity in young children caused the premature closure of distal radial growth plates as evidenced in studies of young gymnasts (Carter and Aldridge, 1988; Albenese et al. 1989). Therefore the introduction of low to moderate intensity exercise may be a safe level of activity necessary to increase the density of an adolescent population.

This study contributes to the knowledge of the effects of physical activity on the BMD and BMC of young males. Previously, information has been limited to that obtained from cross-sectional studies and these studies provide no measure of density prior to participation in their exercise program or sport. This training study has provided evidence that exercise of a moderate intensity may contribute to the maintenance of normal levels of bone mineral density and content. As bone mineral density did not change in those with a normal bone density following the 16 week running program, it would be interesting to investigate the effects of an extended running program of moderate intensity in this same population. Exercise is one contributing lifestyle stimulus that is responsible for changing an individual of low bone density and mass and the effect of other environmental factors such as diet, smoking and alcohol consumption requires further investigation. The moderate intensity running stimulus over the 4 month intervention period did not change BMD or BMC at the sites studied, although the exercise program contributed to the maintenance of these sites and increased cardiovascular fitness.

CHAPTER 6

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The mineralisation of the skeleton begins early in life (Carter, 1987) and develops throughout adolescence until skeletal maturity is reached. In order to attain the greatest bone density and mass at maturity termed peak bone mass, it is suggested that increased physical activity may have a positive effect on the skeleton. In one investigation, male and female long-distance runners were found to have approximatley 40 per cent more bone mineral in the lumbar spine than matched sedentary controls (Lane et al. 1986). Investigations of tennis players have also found bone density to be greater in the dominant compared to their non-dominant arm (Huddleston et al. 1980). Other investigations have shown those involved in activities such as weight-training and swimming have greater density than sedentary individuals (Block et al. 1986; Orwoll et al. 1989). These studies are however limited in their capacity to provide knowledge of subjects' bone mass prior to starting their physical activity. Longitudinal intervention programs have generally focused on postmenopausal women, in light of the common bone loss disorders that affect this population. Few researchers have utilised longitudinal training programs on males. Positive changes in bone density have been reported in middle aged males that participated in a moderate intensity exercise program of 9 months duration (Williams et al. 1984). A program using similar aged subjects and exercise intensity reported a maintenance effect on bone mineral content levels following a 3 month exercise period (Dalen and Olsson, 1974). An intensive exercise regime was associated with changes in bone density of a young adult population following 14 weeks of activity (Leichter et al. 1989). However, the intensive activity was not only responsible for increasing bone density but also the occurrence of stress related injury. The effect of exercise on BMD and BMC is specific to the type, intensity and duration of the activity undertaken. Recently attention has been focused on the importance of peak bone mass, and the interaction of environmental and lifestyle factors such as diet and

exercise, in optimising a young adult's genetic potential to achieve a high bone mass. Participation in exercise may be one stimulus that can be utilised to increase the amount of bone mineral accumulated at maturity and provide a safety margin against age-related bone loss. In this study, an exercise program of moderate intensity was designed on progressive increases in heart rate to increase cardiovascular fitness and examine the effects on bone metabolism, bone mineral density and content and association between bone composition and anthropometric variables. The sites chosen for measurement were the proximal femur and lumbar spine due to their high trabecular content and susceptibility to injury. Therefore the purpose of this investigation was to evaluate the effects of a 16 week running training program on bone composition and bone metabolism in young sedentary males.

Sixteen sedentary males, with an average age of 22.6 years undertook a 16 week program of progressive running training. As the exercise program progressed the running distance became longer and the intensity of the runs was increased. Ten males matched for age, weight and height acted as the control group and were asked to refrain from organised physical activity. Informal activity was recorded by the control group in their activity diary. Members of the exercise group kept a diary of any activity undertaken, including both prescribed and informal activity. Diaries were used to quantify the physical activity histories for each subject over the 16 week period. A maximal exercise treadmill test was conducted to assess the cardiovascular fitness of all subjects and establish the training intensities for the exercising subjects, before commencement of the program. This test was repeated at 4 weekly intervals with the final assessment in week 16. Cumulative heart rates from the maximal exercise tests and time to exhaustion on the treadmill were used as indicators of a change in cardiovascular fitness over the 16 weeks. Bone mineral density and content was assessed at three sites on the proximal femur and in the 2nd to 4th lumbar vertebrae using dual energy x-ray absorptiometry (Norland XR-26). The biochemical markers of bone formation and resorption, namely serum osteocalcin and urinary hydroxyproline were used to evaluate the effects of the exercise program on bone

metabolism. Correlation coefficients were obtained to assess any relationship between cardiovascular fitness and BMD or BMC. Associations between anthropometric variables and BMD or BMC were also investigated.

Analysis of the pre test scores for all measures revealed there were no significant differences between the exercise and non-exercise group. When the mean difference between the pre and post test scores were examined BMD and BMC did not change at the majority of skeletal sites in the exercise group following the 16 week exercise program. The one exception to this result occurred at the 4th lumbar vertebrae where a significant decrease in BMD and BMC was shown in the exercise group following the running program. An increase in BMD of 15 per cent was recorded at the femoral neck by one subject who was identified as having the lowest density of the group at all sites prior to the exercise program. This subject was also found to have elevated levels of both bone markers, urinary hydroxyproline and serum osteocalcin. No significant difference was found for levels of urinary hydroxyproline and serum osteocalcin following the exercise program between the exercise and non-exercise group. There was normal individual variation in the exercising subjects' values for both osteocalcin and hydroxyproline and all subjects were within clinical ranges. A trend towards a decrease was evident in both bone markers for the non-exercise group. Bone mineral density and content were not correlated with either urinary hydroxyproline or serum osteocalcin.

The running program had a significant effect on the cardiovascular fitness of the experimental group, with a significant decrease in cumulative heart rate and a 12% increase in time to exhaustion on the treadmill. Cardiovascular fitness was not significantly correlated with either BMD or BMC following the intervention period. Significant correlations were observed between weight and BMD/BMC, height and BMD/BMC post exercise, although this did occur not for all skeletal sites. Height was found to be the best predictor of BMD and BMC in a stepwise multiple regression procedure.

The lack of a change in BMD and BMC was consistent with earlier investigations that utilised moderate intensity intervention which ranged from 3-12 months (Dalen and Olsson, 1974; Cavanaugh et al. 1988; Nelson et al. 1991). The running program did not increase or decrease bone composition in either exercise or control subjects in the majority of skeletal sites investigated. The exception was L4 which showed a decrease in both BMD and BMC. The control subjects were in fact not truly sedentary as they participated in an average of approximately 1.2 hours/week of informal activity. The exercise group participated in approximately 6 hours/week of both formal and informal activity. This result is an indication of the broad range of mechanical loading that satisfies the maintenance rather than the acquisition of bone mineral density and content.

Summary Interpretation

The results from this thesis indicate the existence of threshold ranges of mechanical usage. The activity undertaken by both the exercise and control groups during the intervention period may be below the threshold ranges that cause bone mineral acquisition. This may be due to genetically adopted "set points" that control threshold ranges (Frost, 1993). The running program did however increase mechanical usage sufficiently so as subsequent strains were not at the bottom of the threshold range, thereby maintaining bone mineral density and content.

Conclusions

On the basis of the findings from this study the following conclusions may be drawn:

(i) There was no change in bone mineral density or bone mineral content at the majority of skeletal sites following the 16 week running program. The exception was a decrease in bone mineral density and bone mineral content at the 4th lumbar vertebrae.

(ii) The exercise intervention increased bone mineral density at the femoral neck by 15 per cent in one subject who began the experiment with the lowest density measures at all sites.

(iii) There was no change in serum osteocalcin or urinary hydroxyproline in the exercising subjects following the 16 week running program.

(iv) Height was found to be the most significant predictor of bone mineral density and bone mineral content in young males.

(v) There was a significant increase in cardiovascular fitness following the 16 week running program in exercising subjects and no association between cardiovascular fitness and bone mineral density or bone mineral content.

Recommendations

The findings of this study have opened several avenues that require investigation in future studies concerning the effects of exercise on bone tissue.

(1) Investigation of the influence of moderate intensity exercise on subjects in the same age group with a below normal bone mineral density, to test the hypothesis that elevation of bone mineral density to normal levels may occur in those with a low bone mineral density prior to exercise.

(2) Repeat the study manipulating the frequency, intensity and duration of the running program using the same population.

(3) Investigate the possibility of changes in the structural geometry of the trabecular network following moderate intensity exercise in young and old populations.

REFERENCES

Albanese, S.A., Palmer, A.K., Kerr, D.R., Carpenter, C.W., Lisi, D. & Levinsohn, E.M. (1989) Wrist pain and distal growth plate closure of the radius in gymnasts. Journal of Pediatric Orthopaedics. 9(1): 23-28.

Alho, A., Husby, T. & Hoiseth, A. (1988) Bone mineral content and mechanical strength. Clinical Orthopaedics and Related Research. 227: 292-297.

Aloia, J.F. (1982) Estrogen and exercise in prevention and treatment of osteoporosis. Geriatrics. 37(6): 81-85.

American College of Physicians. (1984) Radiologic methods to evaluate bone mineral content. Health and Public Policy Committee, Annals of Internal Medicine. 100: 908-911.

American College of Physicians. (1987) Bone mineral densitometry. Health and Public Policy Committee, Annals of Internal Medicine. 107: 932-936.

Armstrong, W.D. (1946) Bone growth in paralysed limbs. Proceedings of the Society of Experimental Biology. 61:358-363.

Arai, H., Nagao, K. & Furutachi, M. (1990) The evaluation of three different bone densitometry systems: XR-26, QDR-1000, and DPX. Image Technology and Information Display. 22(18): 1-6.

Bailey, D.A. and McCulloch, R.G. (1992) Osteoporosis: are there childhood antecedents for an adult health problem? The Canadian Journal of Pediatrics. 130-134.

Bass, W.M. (1971) Human osteology. A laboratory and field manual of the human skeleton. Missouri Archaeological Society, University of Missouri Press.

Bevier, W.C., Wiswell, R.A., Pyka, G., Kozak, K.C., Newhall, K.M. & Marcus, R. (1989) Relationship of body composition, muscle strength and aerobic capacity to bone mineral density in older men and women. Journal of Bone and Mineral Research, 4(3): 421-432.

Bilanin, J.E., Blanchard, M.S. & Russek-Cohen, E. (1989) Lower vertebral bone density in male long distance runners. Medicine and Science in Sports and Exercise. 21(1): 66-70.

Block, J.E., Freidlander, A.L., Brooks, G.A., Steiger, P., Stubbs, H.A. & Genant H.K. (1989) Determinants of bone mineral density among athletes engaged in weight-bearing activity. Journal of Applied Physiology. 67(3): 1100-1105.

Block, J.E., Genant, H.K. & Black, D. (1986) Greater vertebral bone mineral mass in exercising young men. West Journal of Medicine. 145: 39-42.

Bonjour, J.P., Theintz, G., Buchs, B., Slosman, D. & Rizzoli, R. (1991) Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. The Journal of Clinical Endocrinology and Metabolism. 73(3): 555-563.

Boobis, L.H., Hardman, A.E., Williams, C. & Wood, P.C. (1984) Influence of single leg training on submaximum endurance and muscle metabolism in man. Journal of Physiology. 349: 40P.

Boyd-Eaton, S. and Nelson, D.A. (1991) Calcium in evolutionary perspective. American Journal of Clinical Nutrition. 54: S281-S287.

Brouha, L., Smith, P.E., De Lanne, R. & Maxfield, M.E. (1961) Physiological reactions of men and women during muscular activity and recovery in various environments. Journal of Applied Physiology. 16(1): 133-140.

Brown, J.P., Delmas, P.D., Malaval, L., Edouard, C., Chapuy, M.C., & Meunier, P.J. (1984) Serum bone GLA-protein: A specific marker for bone formation in postmenopausal osteoporosis. The Lancet. 1091-1093.

Buskirk, E.R., Iampietro, P.F. & Bass, D.E. (1958) Work performance after dehydration: Effects of physical conditioning and heat acclimatisation. Journal of Applied Physiology. 12: 189-194.

Carter, S.R. and Aldridge, M.J. (1988) Stress injury of the distal radial growth plate. The Journal of Bone and Joint Surgery. 70-B: 834-836.

Carter, D.R., Orr, T.E., Fyhrie, D.P. & Schurman, D.J. (1987) Influences of mechanical stress on prenatal and postnatal skeletal development. Clinical Orthopaedics and Related Research. 219: 237-250.

Carter, D.R. and Spengler, D.M. (1978) Mechanical properties and composition of cortical bone. Clinical Orthopaedics and Related Research. 135: 192-217.

Catherwood, B.D., Marcus, R., Madvig, P. & Cheung, A.K. (1985) Determinants of bone gamma-carboxyglutamic acid-containing protein in plasma of healthy subjects. Bone. 6: 9-13.

Cavanaugh, D.J. and Cann, C.E. (1988) Brisk walking does not stop bone loss in postmenopausal women. Bone. 9: 201-204.

Chamay, A. and Tschantz, P. (1972) Mechanical influences in bone remodelling: Experimental research on Wolfs law. Journal of Biomechanics. 5:173-180.

Cheng, S., Suominen, H., Rantanen, T. & Heikkinen, E. (1990) Bone mineral density and physical activity in 50-60 year old women. Proceedings of the 24th International Federation of Sports Medicine. Amsterdam, May.

Chow, R., Harrison, J. & Notarius, C. (1987) Effect of two randomised exercise programmes on bone mass of healthy postmenopausal women. British Medical Journal. 295: 1441-1444.

Chow, R.K., Harrison, J.E., Brown, C.F. & Hajek, V. (1986) Physical fitness effect on bone mass in postmenopausal women. Archives of Physical Medicine and Rehabilitation. 67: 231-234.

Cole, D.E., Carpenter, T.O. & Gundberg, C.M. (1985) Serum osteocalcin concentrations in children with metabolic bone disease. The Journal of Pediatrics. 106: 770-776.

Compston, J.E., Mellish, R.W.E., Croucher, P., Newcombe, R. & Garrahan, N.J. (1989) Structural mechanisms of trabecular bone loss in man. Bone and Mineral. 6: 339-350.

Dalen, N. and Olsson, K.E. (1974) Bone mineral content and physical activity, Acta Orthopaedica Scandinavica. 45: 170-174.

Dalsky, G.P., Stocke, K.S., Ehsani, A.A., Slatopolsky, E., Lee, W.C. & Birge, S.J. (1988) Weight-bearing exercise training and lumbar bone mineral content in post-menopausal women. Annals of Internal Medicine. 108: 824-828.

Davies, C.T.M. (1968) Limitations to the prediction of maximum oxygen intake from cardiac frequency measurements. Journal of Applied Physiology. 24(5): 700-706.

Delmas, P.D. (1988) Biochemical markers of bone turnover in osteoporosis. In: Osteoporosis: Etiology, Diagnosis and Management. Edited by Riggs BL and Melton LJ. Raven Press, New York.

Delmas, P.D., Stenner, D., Wahner, H.W., Mann, K.G. & Rigg,s B.L. (1983) Increase in serum bone gamma-carboxyglutamic acid protein with aging in women. Journal of Clinical Investigation. 71: 1316-1321.

Donaldson, C.L., Hulley, S.B., Vogel, J.M., Hattner, R.S., Bayers, J.H. & McMillan, D.E. (1970) Effect of prolonged bed rest on bone mineral. Metabolism. 19(12): 1071-1084.

Drinkwater, B.L. (1993) Exercise in the prevention of osteoporosis. Osteoporosis International. Suppl. 1: S169-171.

Drinkwater, B.L. and Chesnut, C.H. (1991) Bone density changes during pregnancy and lactation in active women: a longitudinal study. Bone and Mineral. 14: 153-160.

Eisman, J.A., Kelly, P.J., Morrison, N.A., Pocock, N.A., Yeoman, R., Birmingham, J. & Sambrook, P.N. (1993) Peak bone mass and osteoporosis prevention. Osteoporosis International. Suppl. 1: S56-60.

Epstein, S., Poser, J., McClintock, R., Johnston, C.C., Bryce, G. & Hui, S. (1984) Differences in serum bone gla protein with age and sex. The Lancet. 1: 307-310.

Evans, F.G. (1953) Methods of studying the biochemical significance of bone. American Journal of Physical Anthropometry. 11: 413-419.

Felsing, N.E., Brasel, J.A. & Cooper, D.M. (1992) Effect of high and low intensity exercise on circulating growth hormone in men. Journal of Clinical Endocrinology and Metabolism. 75(1): 157-162.

Fisher, E.C., Nelson, M.E., Frontera, W.R., Turksoy, R.N. & Evans, W.J. (1986) Bone mineral content and levels of gonadotropins and estrogens in amenorrheic running women. Journal of Clinical Endocrinology and Metabolism. 62: 1232-1236.

Forwood, M.R. and Parker, A.W. (1991) Repetitive loading in vivo of the tibiae and femora of rats: effects of repeated bouts of treadmill running. Bone and Mineral. 13: 35-46.

Forwood, M.R. and Parker, A.W. (1989) Microdamage in response to repetitive tortional loading in the rat tibia. Calcified Tissue International. 45(1): 47-53.

Forwood, M.R. and Parker, A.W. (1987) Effects of exercise on bone growth: mechanical and physical properties studied in the rat. Clinical Biomechanics. 2: 185-190.

Frost, H.M. (1993) Suggested fundamental concepts in skeletal physiology. Calcified Tissue International. 52: 1-4.

Frost, H.M. (1987) The mechanostat: a proposed pathologic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. Bone and Mineral. 2: 73-85.

Frost, H.M. (1983) A determination of bone architecture: The minimum effective strain. Clinical Orthopeadics and Related Research. 175: 286-292.

Geusens, P., Dequeker, J., Nijs, J., Verstraeten, A. & Bramm, E. (1986) Age, sex and menopause-related changes of vertebral and peripheral bone: population study using dual and single photon absorptiometry and radiogrammetry. Journal of Nuclear Medicine. 27, 1540-1549.

Geusens, P., Cantatore, F., Nijis, J., Proesmans, W., Emma, F. & Dequeker, J. (1991) Heterogeneity of growth of bone in children at the spine, radius and total skeleton. Growth, Development and Aging. 55: 249-256.

Gilsanz, V., Gibbens, D.T., Carlson, M., Ines Boechat, M., Cann, E. & Schulz, E.E. (1988) Peak trabecular vertebral density: A comparison of adolescent and adult females. Calcified Tissue International. 43: 260-262.

Gilsanz, V., Gibbens, D.T., Roe, T.F., Carlson, M., Senac, M.O., Ines Boechat, M., Haung, H.K., Schulz, E.E., Libanati, C.R. & Cann, C.C. (1988) Vertebral bone density in children: Effect of puberty. Radiology. 166: 847-850.

Glastre., C., Braillon, P., David, L., Cochat, P., Meunier, P.J. & Delmas, P.D. (1990) Measurement of bone mineral content of the lumbar spine by dual energy x-ray absorptiometry in normal children: Correlations with growth parameters. Journal of Clinical Endocrinology and Metabolism. 70(5): 1330-1333.

Goodship, A.E., Lanyon, L.E. & McFie, H. (1979) Functional adaptation of bone to increased stress. The Journal of Bone and Joint Surgery. 61-A(4): 539-546.

Gordon, C.L., Halton, J.M. & Atkinson, S.A. (1991) The contributions of growth and puberty to peak bone mass. Growth, Development and Aging. 55: 257-262.

Gotfredsen, A., Hadberg, A., Nilas, L. & Christiansen, C. (1987) Total body bone mineral in healthy adults. Journal of Laboratory Clinical Medicine. 110: 362-368.

Gotfredsen, A., Borg, J., Christiansen, C. & Mazess, R.B. (1984) Total body bone mineral in vivo by dual photon absorptiometry. I. Measurement procedures. Clinical Physiology. 4: 343-355.

Granhed, H., Jonson, R. & Hansson, T. (1989) Mineral content and strength of lumbar vertebrae. A cadaver study. Acta Orthopaedica Scandinavica. 60: 105-109.

Griffith, R.O., Dressendorfer, R.H., Fullbright, C.D. & Wade, C.E. (1990) Testicular function during exhaustive endurance training. The Physician and Sportsmedicine. 18(5): 54-64.

Hackney, A.C., Sinning, W.E. & Bruot, B.C. (1988) Reproductive hormonal profiles of endurance-trained and untrained males. Medicine and Science in Sports and Exercise. 20(1): 60-65.

Hardman, A.E., Williams, C. & Wootton, S.A. (1986) The influence of short-term endurance training on maximum oxygen uptake, submaximum endurance and the ability to perform brief, maximal exercise. Journal of Sports Sciences. 4: 109-116.

Harris, S. and Dawson-Hughes, B. (1992) Rates of change in bone mineral density of the spine, heel, femoral neck and radius in healthy postmenopausal women. Bone and Mineral. 17: 87-95.

Hayes, W.C. (1991) Biomechanics of cortical and trabecular bone: Implications for assessment of future risk. In: Basic Orthopaedic Biomechanics, Edited by Mow VC and Hayes WC. Raven Press Ltd, New York.

Hopwood,. J.J. and Harrison, J.R. (1982) High-resolution electrophoresis of urinary glycosaminoglycans: An improved screening test for the mucopolysaccharidoses. Analytical Biochemistry. 119:120-127.

Huddleston, A.L., Rockwell, D., Kulund, D.N. & Harrison, B. (1980) Bone mass in lifetime tennis athletes. Journal of the American Medical Association. 244(10): 1107-1109.

Jaworski, Z.F.G. (1976) Parameters and indices of bone resorption. In: Bone Histomorphometry, Edited by Meunier PJ, 2nd International Workshop, Lyon (Armour Montague.

Jee, W.S.S., Wronski, T.J., Morey, E.R. & Kimmel, D.B. (1983) Effects of spaceflight on trabecular bone in rats. American Journal of Physiology. 244: R310-R314.

Jones, H.H., Preist, J.D., Hayes, W.C., Tichenor, C.C. & Nagel, D.A. (1977) Humeral hypertrophy in response to exercise. Journal of Bone and Joint Surgery. 59-A(2): 204-208.

Kelly, P.J., Eisman, J.A., Stuart, M.C., Pocock, N.A., Sambrook, P.N. & Gwinn, T.H. (1990) Somatomedin-C, physical fitness, and bone density. Journal of Clinical Endocrinology and Metabolism. 70(3): 718-723.

Kelly, P.J., Pocock, N.A., Sambrook, P.N., & Eisman, J.A. (1989) Age and menopause-related changes in indices of bone turnover. Journal of Clinical Endocrinology and Metabolism. 69(6): 1160-1165.

Kelly, T.L., Slovik, D.M., Schoenfeld, D.A. & Neer, R.M. (1988) Quantitative digital radiography versus dual photon absorptiometry of the lumbar spine. The Journal of Clinical Endocrinology and Metabolism. 67(4): 839-844.

Kiiskinen, A. (1977) Physical training and connective tissues in young mice. Physical properties of achillies tendons and long bones. Growth. 41: 123-137.

Krabbe, S. and Christiansen, C. (1984) Longitudinal study of calcium metabolism in male puberty. Acta Pediatrica Scandinavica. 73: 745-749.

Krabbe, S., Christiansen, C., Rodbro, P. & Transbol, J. (1979) Effect of puberty rates on bone growth and mineralisation: With observations in male delayed puberty. Archives of Disease in Childhood. 45: 950-953.

Kraemer, R.R., Kilgore, J.L., Kraemer, G.R. & Castracane, V.D. (1992) Growth hormone, IGF-I, and testosterone responses to resistive exercise. Medicine and Science in Sports and Exercise. 24(12): 1346-1352.

Kriska, A.M., Black, Sandler. R., Cauley, J.A., LaPorte, R.E., Hom, D.L. & Pambianco, G. (1988) The assessment of historical physical activity and its relation to adult bone parameters. American Journal of Epidemiology. 127(5): 1053-1061.

Kroger, H., Kotaniemi, A., Vainio, P. & Alhava, E. (1992) Bone densitometry of the spine and femur in children by dual-energy x-ray absorptiometry. Bone and Mineral. 17: 75-85.

Krolner, B. and Toft, B. (1983) Vertebral bone loss: an unheeded side effect of therapeutic bed rest. Clinical Science. 64: 537-540.

Krolner, B., Pors Nielson, S., Lund, B., Lund, B.J., Sorensen, O.H. & Uhrenholdt, A. (1980) Measurement of bone mineral content (BMC) of the lumbar spine, II. Correlation between forearm BMC and lumbar spine BMC. Scandinavian Journal of Clinical and Laboratory Investigation. 40: 665-670.

Laitinen, K., Valimaki, M. & Keto, P. (1991) Bone mineral density measured by dual-energy x-ray absorptiometry in healthy finnish women. Calcified Tissue International. 48: 224-231.

Lane, N.E., Bloch, D.A., Wood, P.D. & Fries, J.F. (1987) Aging, long-distance running, and the development of musculoskeletal disability. The American Journal of Medicine. 82: 772-780.

Lanyon, L.E. and Rubin, C.T. (1984) Static vs dynamic loads as an influence on bone remodelling. Journal of Biomechanics. 17(12): 897-905.

Lanyon, L.E., Hampson, G.J., Goodship, A.E. & Shah, J.S. (1975) Bone deformation recorded in vivo from strain gauges attached to the human tibial shaft. Acta Orthopaedica Scandinavica. 46: 256-268.

Lanyon, L.E. (1973) Analysis of surface bone strain in the calcaneus of sheep during normal locomotion. Journal of Biomechanics. 6:41-49.

Lanyon, L.E. (1972) In vivo bone strain recorded from thoracic vertebrae of sheep. Journal of Biomechanics. 5: 277-281.

Lanyon, L.E. (1971) Strain in sheep lumbar vertebrae recorded during life. Acta Orthopaedica Scandinavica. 42, 102-112.

Leichter, I., Simkin, A., Margulies, J.Y., Bivas, A., Steinberg, R., Giladi, M. & Milgrom, C. (1989) Gain in mass density of bone following strenuous physical activity. Journal of Orthopaedic Research. 7: 86-90.

Li, K-C., Zernicke, R.F., Barnard, J. & Li, A. F-Y. (1991) Differential response of rat limb bones to strenuous exercise. Journal of Applied Physiology. 70(2): 554-560.

Li, X.J., Jee, W.S.S., Chow, Y. & Woodbury, D.M. (1990) Adaptation of cancellous bone to aging and immobilisation in the rat: A single photon absorptiometry and histomorphometry Study. The Anatomical Record. 227:12-24.

Lian, J.B. and Friedman, P.A. (1978) The vitamin k-dependent synthesis of gamma-carboxyglutamic acid by bone microsomes. The Journal of Biological Chemistry. 253(19): 6623-6626.

MacDougall, J.D., Webber, C.E., Martin, J., Ormerod, S., Chesley, A., Younglai, E.V., Gordon, C.L. & Blimkie, C.J.R. (1992) Relationship among running mileage, bone density, and serum testosterone in male runners. Journal of Applied Physiology. 73(3): 1165-1170.

McCarthy, R.N. and Jeffcott, L.B. (1992) Effects of treadmill exercise on cortical bone in the third metacarpus of young horses. Research in Veterinary Science. 52: 28-37.

McElduff, A., Wilkinson, M., Ward, P. & Posen, S. (1988) Forearm mineral content in normal men: Relationship to weight, height and plasma testosterone concentrations. Bone. 9:281-283.

Mack, J.M., Henken, E.M. & Spencer, R.P. (1989) Nuclear medicine studies of aging--VII. Humeral deltoid tuberosity on bone scans and radiographs. International Journal of Radiation and Applied Instrumentation. 16(5): 469-472.

Mack, P.B., LaChance, P.A., Vose, G.P. & Vogt, F.B. (1967) Bone demineralisation of foot and hand of gemini-titan iv, v and vii astronauts during orbital flight. The American Journal of Roengenology Radium Therapy and Nuclear Medicine. 100(3): 503-511.

Margulies, J.Y., Simkin, A., Leichter, I., Bivas, A., Steinberg, R., Giladi, M., Stein, M., Kashtan, H. & Milgrom, C. (1986) Effect of intense physical activity on the bone mineral content in the lower limbs of young adults. The Journal of Bone and Joint Surgery. 68-A(7): 1090-1093.

Martin, R.B. and Burr, D.B. (1989) Structure, Function and Adaptation of Compact Bone. Raven press, New York.

Matkovic, V. (1992) Calcium intake and peak bone mass. The New England Journal of Medicine. 327(2): 119-120.

Matsuda, J.J., Zernicke, R.F., Vailas, A.C., Pedrini, V.A., Pedrini-Mille, A. & Maynard, J.A. (1986) Structural and mechanical adaptation of immature bone to strenuous exercise. Journal of Applied Physiology. 60(6): 2028-2034.

Mazess, R., Collick, B., Trempe, J., Barden, H., & Hanson, J. (1989) Performance evaluation of a dual-energy x-ray bone densitometer. Calcified Tissue International. 44: 228-232.

Melton, L.J., Eddy, D.M. & Johnston, C.C. (1990) Screening for osteoporosis. Annals of Internal Medicine. 112: 516-528.

Meier, D.E., Orwoll, E.S. & Jones, J.M. (1984) Marked disparity between trabecular and cortical bone loss with age in healthy men. Annals of Internal Medicine. 101: 605-612.

Michel, B.A., Bloch, D.A. & Fries, J.F. (1989) Weight-bearing exercise, overexercise and lumbar bone density over age 50 years. Archives of Internal Medicine. 149: 2325-2329.

Miller, J.Z., Slemenda, C.W., Meaney, F.J., Reister, T.K., Hui, S. & Johnson, C.C. (1991) The relationship of bone mineral density and anthropometric variables in healthy male and female children. Bone and Mineral. 14: 137-152.

Myburgh, K.H., Hutchins, J., Fataar, A., Hough, S.F. & Noakes, T.D. (1990) Low bone density is an etiologic factor for stress fractures in athletes. Annals of Internal Medicine. 113(10): 755-759.

Nelson, M.E., Fisher, E.C., Dilmanian, F.A., Dallal, G.E. & Evans, W.J. (1991) A one year walking program and increased dietary calcium in postmenopausal women: effects on bone. American Journal of Clinical Nutrition. 53: 1304-1311.

Nichols Institute Diagnostics. (1991) Human Osteocalcin Radioimmunoassay Kit Manual, San Juan Capistrano.

Nilsson, B.E. and Westlin, N.E. (1971) Bone density in athletes. Clinical Orthopeadics and Related Research. 77(6): 179-182.

Nishimoto, S.K. and Price, P.A. (1979) Proof that the gamma-carboxyglutamic acid-containing bone protein is synthesised in calf bone. The Journal of Biological Chemistry. 254(2): 437-441.

Nordesjo, L.O. (1974) The effect of quantitated training on the capacity for short and prolonged work. Acta Physiologica Scandinavica. S40-45.

Nordin, B.E.C., Horsman, A. & Aaron, J. (1976) In: Calcium, Phosphate and Magnesium Metabolism. Edited by Nordin BEC, pp. 469-524, Churchill Livingstone, New York.

Norris, R., Carroll, D. & Cochrane, R. (1990) The effects of aerobic and anaerobic training on fitness, blood pressure, and psychological stress and well-being. Journal of Psychosomatic Research. 34(4): 367-375.

Nuti, R. and Martini, G. (1992) Measurements of bone mineral density by DXA total body absorptiometry in different skeletal sites in postmenopausal osteoporosis. Bone. 13: 173-178.

Organo Teknika. (1989) Hpyronostican Manual, Boxtel Holland.

Ormerod, S., MacDougall, D., Webber, C., Martin, J. & Blimkie, J. (1990) The relationship between weekly mileage and bone density in male runners. Medicine and Science in Sports and Exercise. 22(2): S62.

Orwoll, E.S., Ferar, J., Oviatt, S.K., McClung, M.R. & Huntington, K. (1989) The relationship of swimming exercise to bone mass in men and women. Archives of Internal Medicine. 149(10): 1297-2200.

Parfitt, A.M. (1988) Bone remodeling: Relationship to the amount and structure of bone, and the pathogenesis and prevention of fractures. In: Osteoporosis: Etiology, Diagnosis and Management. Edited by, Riggs BL. and Melton LJ. pp.45-94, Raven press, New York.

Parfitt, A.M. (1980) Morphologic basis of bone mineral measurements: Transient and steady state effects of treatment in osteoporosis. Mineral Electrolyte Metabolism. 4: 273-287.

Patterson-Buckendahl, P.E., Grindeland, R.E., Martin, R.B., Cann, C.E. & Arnaud, S.B. (1985) Osteocalcin as an indication of bone metabolism during spaceflight. The Physiologist. 28(6): S-227-S228.

Peterson, S.E., Peterson, M.D., Raymond, G., Gilligan, C., Checovich, M.M., & Smith, E.L. (1991) Muscular strength and bone density with weight training in middle-aged women. Medicine and Science in Sports and Exercise. 23(4): 499-504.

Picard, D., Ste-Marie, L.G., Coutu, D., Carrier, L., Chartrand, R., Lepag,e R., Fugere, P. & D'Amour, P. (1988) Premenopausal bone mineral content relates to height, weight and calcium intake during early adulthood. Bone and Mineral. 4: 299-309.

Pocock, N., Eisman, J., Gwinn, T., Sambrook, P., Kelly, P., Freund, J. & Yeates, M. (1989) Muscle strength, physical fitness and weight but not age predict femoral neck bone mass. Journal of Bone and Mineral Research. 4(3): 441-443.

Pocock, N.A., Eisman,J.A., Hopper, J.L., Yeates, M.G., Sambrook, P.N. & Eberi, S. (1987) Genetic determinants of bone mass in adults. Journal of Clinical Investigation. 80: 706-710.

Pocock, N.A., Eisman, J.A., Yeates, M.G., Sambrook, P.N. & Ebert, S. (1986) Physical fitness is a major determinant of femoral neck and lumbar spine bone mineral density. Journal of Clinical Investigation. 78: 618-621.

Price, P.A. and Nishimoto, S.K. (1980) Radioimmunoassay for the vitamin k-dependent protein of bone and its discovery in plasma. Proceedings of the National Academy of Sciences USA. 77(4): 2234-2238.

Rasmussen, H. and Bordier, P. (1974) The Physiological and Cellular Basis of Bone Disease. p.19, Williams and Wilkins Co., Baltimore.

Resch, H., Pietschmann, P., Woloszczuk, W., Krexner, E. & Bernecker, P. (1992) Bone mass and biochemical parameters of bone metabolism in men with spinal osteoporosis. European Journal of Clinical Investigation. 22: 542-545.

Rico, H., Revilla, M., Hernandez, E.R., Villa, L.F. & Alvarez del Burergo, M. (1992) Sex differences in the acquisition of total bone mineral mass peak assessed through dual-energy x-ray absorptiometry. Calcified Tissue International. 51: 251-254.

Riggs, B.L., Wahner, H.W., Seemen, E., Offord, K.P., Dunn, W.L., Mazess, R.B., Johnson, K.A. & Melton, L.J. (1982) Changes in bone mineral density of the proximal femur and spine with aging. Journal of Clinical Investigation. 70: 716-723.

Riggs, B.L., Wahner, H.W., Dunn, W.L., Mazess, R.B., Offord, K.P. & Melton, L.J. (1981) Differential changes in bone mineral density of the appendicular and axial skeleton with aging. Journal of Clinical Investigation. 67(2): 328-335.

Rowell, L.B., Taylor, H.L. & Wang, Y. (1964) Limitations to prediction of maximal oxygen uptake. Journal of Applied Physiology. 19(5): 919-927.

Rubin, C.T. (1984) Skeletal strain and the functional significance of bone architecture. Calcified Tissue International. 36: S11-S18.

Rubin, C.T. and Lanyon, L.E. (1984) Regulation of bone formation by applied dynamic loads. Journal of Bone and Joint Surgery. 66A: 397-402.

Rudd, E. (1989) Preventive aspects of mobility and functional disability. Scandinavian Journal of Rheumatology. 82: S25-32.

Rundgren, A., Anainsson, A., Ljungberg, P. & Wetterqvist, H. (1984) Effects of a training programme for elderly people on mineral content of the heel bone. Archives of Gerontology and Geriatrics. 3: 243-248.

Russell, R.G.G., Kanis, J.A., Gowen, M., Gallagher, J.A., Beresford, J., Guilland-Cumming, D., Coulton, L.A., Preston, C.J., Brown, B.L., Sharrard, M. & Beard, D.J. (1983) Cellular control of bone formation and repair. In Osteoporosis, A Multi-disciplinary Problem. Edited by A. St. J. Dixon, RGG Russell and TCB Stamp.

Saltin, B., Nazar, K., Costill, D.L., Stein, E., Jansson, E., Essen, B. & Gollnick, P.D. (1976) The nature of the training response; Peripheral and central adaptations to one-legged exercise. Acta Physiologica Scandinavica. 96: 289-305.

Sambrook, P.N. (1991) Osteoporosis: significance, diagnosis, prevention and management. Modern Medicine. 21(8): 22-32.

Sevastikoglou, J.A. and Larsson, S.E. (1976) Changes in composition and metabolic activity of the skeletal parts of the extremity of the adult rat following below knee amputation. Acta Chiroptica Scandinavia. 467: S9-15, 1976.

Simkin, A., Leichter, I., Swiss, A. & Samueloff, S. (1989) The effect of swimming activity on bone architecture in growing rats. Journal of Biomechanics. 22(8/9): 845-851.

Simkin, A., Ayalon, J. & Leichter, I. (1987) Increased trabecular bone density due to bone-loading exercises in postmenopausal osteoporotic women. Calcified Tissue International. 40: 59-63.

Sinaki, M. (1989) Exercise and osteoporosis. Archives of Physical Medicine and Rehabilitation. 70(3): 220-229.

Sinaki, M., Opitz, J.L. & Wahner, H.W. (1974) Bone mineral content: Relationship to muscle strength in normal subjects. Archives of Physical Medicine and Rehabilitation. 55(11): 508-512.

Slemenda, C.W., Miller, J.Z., Hui, S.L., Resiter, T.K. & Johnston, C.C. (1991) Role of physical activity in the development of skeletal mass in children. Journal of Bone and Mineral Research. 6(11): 1227-1233.

Snead, D.B., Weltman, A., Weltman, J.Y., Evans, W.S., Veldhuis, J.D., Varma, M.M., Teates, C.D., Dowling, E.A. & Rogal, A. (1992) Reproductive hormones and bone mineral density in women runners. Journal of Applied Physiology. 72(6): 2149-2156.

Snow-Harter, C., Bouxsein, M., Lewis, B., Charette, S., Weinstein, P. & Marcus, R. (1990) Muscle strength as a predictor of bone mineral density in young women. Journal of Bone and Mineral Research. 5(6): 589-595.

Somers, V.K., Conway, J., Johnston, J. & Sleight, P. (1991) Effects of endurance training on barroreflex sensitivity and blood pressure in borderline hypertension. The Lancet. 337: 1363-1368.

Swaine, I.L., Linden, R.J. & Mary, D. (1992) Indices for detection of changes in cardiorespriatory fitness during exercise training in man. Experimental Physiology. 77: 65-78.

Talmage, R.V., Stinnett, S.S., Landwehr, J.T., Vincent, L.M. & McCartney, W.H. (1986) Age-related loss of bone mineral density in non-athletic and athletic women. Bone and Mineral. 1: 115-125.

Taylor, H.L., Wang, Y., Rowell, L. & Blomqvist, G. (1963) The standardization and interpretation of submaximal and maximal tests of working capacity. Pediatrics. 32: S703-722.

Thomas, K.A., Cook, S.D., Bennett, J.T., Whitecloud, T.S. & Rice, J.C. (1991) Femoral neck and lumbar spine bone mineral densities in a normal population 3-20 years of age. Journal of Pediatric Orthopaedics. 11: 48-58.

Tohme, J.F., Seibel, S.J., Robins, S.P. & Bilezikian, J.P. (1991) Biochemical markers of bone metabolism. Zeitschrift fur Rheumatologie. 50: 133-141.

Van der Wiel, H.E., Lips, P., Netelenbos, J.C., & Hazenberg, G.J. (1991) Biochemical parameters of bone turnover during ten days of bed rest and subsequent mobilization. Bone and Mineral. 13: 123-129.

Vaughan, J. (1981) The Physiology of Bone. Third Edition. Oxford University Press. New York pp. 57-75.

Virvidakis, K., Georgiou, E., Korkotsidis, A., Ntall, K. & Proukakis, C. (1990) Bone mineral content of junior competitive weightlifters. International Journal of Sports Medicine. 11(3): 224-246.

Weinreb, M., Rodan, G.A. & Thompson, D.D. (1989) Osteopenia in the immobilised rat hindlimb is associated with increased bone resorption and decreased bone formation. Bone. 10: 187-194.

Whalen, R.T., Carter, D.R. & Steele, C.R. (1988) Influence of physical activity on the regulation of bone density. Journal of Biomechanics. 21(10): 825-837.

Wheeler, G.D., Wall, S.R., Belcastro, A.N. & Cumming, D.C. (1984) Reduced serum testosterone and prolactin levels in male long distance runners. Journal of the American Medical Association. 252(4): 514-516.

Weiner, J.S. and Louri,e J.A. (1969) Human Biology: A Guide to Field Methods. Blackwell Scientific Publications, Oxford.

Williams, J.A., Wagner, J., Wasnich, R. & Heilbrun, L. The effect of long-distance running upon appendicular bone mineral content. Medicine and Science in Sports and Exercise. 16(3): 223-227.

Wilson, C.R., Fogelman, I., Blake, G.M. & Rodin, A. (1991) The effect of positioning on dual energy x-ray bone densitometry of the proximal femur. Bone and Mineral. 13: 69-76.

Woo, S., Kuei,S.C., Amiel, D., Gomez, M.A., Hayes, W.C., White, F.C. & Akeson, WH. (1981) The effect of prolonged physical training on the properties of long bone: A study of Wolff's law. Journal of Bone and Joint Surgery. 63-A(5): 780-786.

APPENDIX I

THE UNIVERSITY OF WOLLONGONG

HUMAN EXPERIMENTATION ETHICS COMMITTEE

MEMO TO: Mr J. Meerkin

Department/School of Human Movement Science

FROM: Professor J. L. C. Chipman
Chairperson
Human Experimentation Ethics Committee

DATE: 22/6/92

RE: Human Ethics Application HE 92/81

Thank you for your response to the Committee's requirements for the above Human Ethics application.

Your response meets with the requirements of the Committee and your application is now formally approved.

Professor J. L. C. Chipman
Chairperson
Human Experimentation Ethics Committee

cc. Head Department/School

APPENDIX II
UNIVERSITY OF WOLLONGONG
HUMAN MOVEMENT DEPARTMENT
MEDICAL QUESTIONNAIRE

This information will be treated as confidential and will not be released or revealed without your written consent. The data may be used however for statistical or scientific purposes anonymously.

PERSONAL INFORMATION:

Name..... Age.....

Birth date...../...../..... Sex M / F

Address.....

Phone.....(H).....(W)

Emergency Contact:

Name..... Phone.....

MEDICAL/HEALTH INFORMATION:

Your Doctor.....

Date of Last Medical Check...../...../.....

HAVE YOU EVER OR DO YOU HAVE? Circle the correct response

1. Heart trouble.....Yes/No
2. Frequent pains in your heart and chest.....Yes/No
3. Felt faint or have spells of severe dizziness.....Yes/No
4. High blood pressure.....Yes/No
5. A bone or joint problem which may be made worse with exercise.....Yes/No
6. Is there a good physical reason not mentioned here why you should not follow an activity program even if you wanted to?.....Yes/No

DO YOU EXPERIENCE OR HAVE YOU EXPERIENCED?

1. A family history of heart disease or stroke of relatives under 65 years of age....Yes/No
2. Breathing difficulties or asthma.....Yes/No
3. Arthritis.....Yes/No
4. A Hernia.....Yes/No
5. Epilepsy.....Yes/No
6. Diabetes.....Yes/No
7. Back Pain.....Yes/No
8. Muscular Pain / Cramps.....Yes/No

APPENDIX II (cont)

9.Do you smoke cigs / pipe / cigar .....Yes/No
If so, how many per day / per week?
.....
.

10. Do you drink alcohol?.....Yes/No
If so, how much per day / per week?
.....
.

11. Have you been hospitalised recently?.....Yes/No
Details.....
.....

12. Do you have or have you recently had any infections or infectious disease?.....Yes/No
Details.....
.....

13. Are there any other conditions that may limit your participation in this study.....Yes/No
Details.....

APPENDIX III
ACTIVITY QUESTIONNAIRE

1. Have you participated in an organised or self program of exercise (> 3 times/week @ > 30 min) within the last five years

.....

2. If yes, what specific activity type did you participate in (e.g.swimming,basketball, tennis) .....

.....

3. Are you currently participating in an organised or self program of exercise; if yes what type of program .....

.....

4. Do you participate in any weight-bearing exercise (Eg. running, walking, cycling)
   If yes, how many hours per week in total .....

USE THIS SPACE TO INCLUDE ANY RELEVENT INFORMATION THAT WE HAVE NOT AS YET GAINED FROM YOU

Medication, Injuries, Problems etc.

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APPENDIX IV

Subject Information Package
Department of Human Movement Science

SUBJECT INFORMATION PACKAGE

THE RESPONSE OF TRABECULAR BONE TO PHYSICAL ACTIVITY IN YOUNG SEDENTARY MALES

ITEM 1: PROJECT OBJECTIVES

The objective of this study is to investigate whether a program of exercise of sufficient time to increase cardiorespiratory fitness will increase the bone mineral density of young sedentary males.

ITEM 2: RATIONALE

Bone loss and bone loss disorders are of major concern and effect all individuals during part of their life. Factors contributing to bone loss and bone disorders include diet, physiological changes in hormone secretion, lack of physical activity and aging. Exercise or physical activity in particular is known to have a positive effect on bone density. The mechanical stress or loads produced by activity promotes the deposition of new bone. However the difficulty arises, as the older the individual becomes the more difficult it is to begin an exercise program that will provide benefit and elevate bone mass. The amount of load bearing exercise needed to induce a change in bone mass is still uncertain. Therefore by assessing a program of exercise with a known duration in a young population it may be possible evaluate the required time to induce changes in bone mass.

ITEM 3: TEST PROCEDURES

As subjects you will undertake a program of running training for a period of 16 weeks. The training program is a graduated program beginning with 20 minute runs and for the last 4 weeks increasing to 40-45 minutes. Runs will be undertaken 3 times per week.

APPENDIX IV (cont)

Subject Information Package
Department of Human Movement Science

Before the beginning of the training program you will undergo a maximal fitness test on a treadmill. Three maximal exercise tests will occur during the training period and another following the end of the 16 weeks training in order to measure the benefits of the training program undertaken.

Three additional measures will be taken during this experiment. These tests will be undertaken before the training program is to begin, and repeated post-training .

The first is a bone density scan of the lumbar spine and proximal femur, using dual energy x-ray absorptiometry. This will take approximately 30 minutes. The following two tests are biochemical tests to measure changes in bone metabolism. They are urinary hydroxyproline which requires you to provide a urine sample and serum osteocalcin which requires a small blood sample. These two tests only require you to show up at the laboratory to provide the sample.

ITEM 4: RISKS AND DISCOMFORTS

Exercise represents a stress to the body, and as such is not without risk, no matter how light the exercise. The major risk factor associated with exercise is cardiovascular dysfunction, which in the worst case may result in coronary arrest. The risks during this experiment are greatest during training as you reach your maximum intensity, and during the sequela maximal exercise tests (performed each month during the protocol). However the probability of a cardiovascular incident is minimal in normal healthy, physically active subjects under 40 years of age.

All subjects will be screened using the Par-Q questionnaire to identify and eliminate subjects likely to be at risk of cardiovascular disorders during exercise. This questionnaire has been medically validated on more than 1200 subjects within the ages of 20-65 years.

The bone density scan to be undertaken by the subjects is a non-invasive procedure. The radiation dose of the scan is much less than that of a normal chest x-ray. In any protocol that requires the donation of serum there is the discomfort of injections, however this will be kept to a minimum and carried out by trained personnel under medical supervision.

APPENDIX IV (cont)

Subject Information Package
Department of Human Movement Science

ITEM 5: INQUIRES

Questions concerning the procedures and/or rationale used in this investigation are welcome at any time. Please ask for clarification of any point which you feel is not explained to your satisfaction. Your initial contact person is the investigator conducting this project. Subsequent inquiries may be directed to Dr Peter Milburn (Head of Department of Human Movement Science: phone 213881).

ITEM 6: FREEDOM OF CONSENT

Participation in this project is entirely voluntary. You are free to deny consent before or during the experiment. In the latter case such withdrawal of consent should be performed at the time you specify, and not at the end of a particular trial. Your participation and/or withdrawal of consent will not influence your present and/or future involvement with the University of Wollongong. In the case of student involvement , it will not influence grades awarded by the University. You have the right to withdraw from any experiment, and the right shall be preserved over and above the goals of this experiment.

ITEM 7: CONFIDENTIALITY

All questions, answers and results of this study will be treated with absolute confidentiality. Subjects will be identified in the resultant manuscripts, reports or publications by the use of subject codes only.

APPENDIX V

Subject Information Package
Department of Human Movement Science

SUBJECT INFORMATION PACKAGE

THE RESPONSE OF TRABECULAR BONE TO PHYSICAL ACTIVITY IN YOUNG SEDENTARY MALES

ITEM 1: PROJECT OBJECTIVES

The objective of this study is to investigate whether a program of exercise of sufficient time to increase cardiorespiratory fitness will increase the bone mineral density of young sedentary males.

ITEM 2: RATIONALE

Bone loss and bone loss disorders are of major concern and effect all individuals during part of their life. Factors contributing to bone loss and bone disorders include diet, physiological changes in hormone secretion, lack of physical activity and aging. Exercise or physical activity in particular is known to have a positive effect on bone density. The mechanical stress or loads produced by activity promotes the deposition of new bone. However the difficulty arises, as the older the individual becomes the more difficult it is to begin an exercise program that will provide benefit and elevate bone mass. The amount of load bearing exercise needed to induce a change in bone mass is still uncertain. Therefore by assessing a program of exercise with a known duration in a young population it may be possible evaluate the required time to induce changes in bone mass.

ITEM 3: TEST PROCEDURES

As subjects three tests will be undertaken during this experiment. These tests will be repeated in 16 weeks time following the first series of tests.

The first is a bone density scan of the lumbar spine and proximal femur, using dual energy x-ray absorptiometry. This will take approximately 30 minutes. The second tests are biochemical tests to measure changes in bone metabolism. They are urinary hydroxyproline which requires you to provide a urine sample and serum osteocalcin which requires a 10 ml blood sample. These two tests only require you to show up at the laboratory to provide the sample. The third test involves a maximal exercise test on the

treadmill where the subject will run at a set speed while the incline on the treadmill rises. Associated with this test is a measure of height and weight. These fitness tests will take approximately 15 minutes.

ITEM 4: RISKS AND DISCOMFORTS

Exercise represents a stress to the body, and as such is not without risk, no matter how light the exercise. The major risk factor associated with exercise is cardiovascular dysfunction, which in the worst case may result in coronary arrest. The risks during this experiment are greatest during training as you reach your maximum intensity. However the probability of a cardiovascular incident is minimal in normal healthy, physically active subjects under 40 years of age.

All subjects will be screened using the Par-Q questionnaire to identify and eliminate subjects likely to be at risk of cardiovascular disorders during exercise. This questionnaire has been medically validated on more than 1200 subjects within the ages of 20-65 years.

The bone density scan to be undertaken by the subjects is a non-invasive procedure. The radiation dose of the scan is much less than that of a normal chest x-ray. In any protocol that requires the donation of serum there is the discomfort of injections, however this will be kept to a minimum and carried out by trained personnel under medical supervision.

ITEM 5: INQUIRES

Questions concerning the procedures and/or rationale used in this investigation are welcome at any time. Please ask for clarification of any point which you feel is not explained to your satisfaction. Your initial contact person is the investigator conducting this project. Subsequent inquiries may be directed to Dr Peter Milburn (Head of Department of Human Movement Science: phone 213881).

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withdrawal of consent will not influence your present and/or future involvement with the University of Wollongong. In the case of student involvement , it will not influence grades awarded by the University. You have the right to withdraw from any experiment, and the right shall be preserved over and above the goals of this experiment.

ITEM 7: CONFIDENTIALITY

All questions, answers and results of this study will be treated with absolute confidentiality. Subjects will be identified in the resultant manuscripts, reports or publications by the use of subject codes only.

APPENDIX VI
Informed Consent

The researcher conducting this project supports the principles governing the ethical conduct of research, and the protection at all times of the interests, comfort and safety of subjects.

This form and the accompanying Subject Information Package are given to you for your own protection. They contain a detailed outline of the experimental procedures, and possible risks. Your signature below indicates six things:

(1) you have received the Subject Information Package;

(2) you have read its contents;

(3) you have been given the opportunity to discuss its contents with one of the researchers prior to commencing the experiment;

(4) you clearly understand these procedures and possible risks;

(5) you voluntarily agree to participate in this project; and

(6) your participation may be terminated at any point in time without jeopardising your involvement with the University of Wollongong, or your course assessment through the University.

Any enquires or further questions may be initially directed to the researcher Mr Jarrod Meerkin on 213881, Dr Nigel Taylor (Supervisor; 042 214094) or to the Head of Department of Human Movement Science: phone 21-3881. Any complaints regarding the conduct of the research may be directed to the Secretary of the University of Wollongong Human Experimentation Ethics Committee on (042) 213079.

I agree to participate in experimental procedures set out in the Subject Information Package.

Last Name:_____ Given Name:_____

Date of Birth: ___/___/___

Address:_____

_____

Name and number of contact person in case of an emergency:

Name:_____ Phone:_____

Family Doctor:_____ Phone:_____

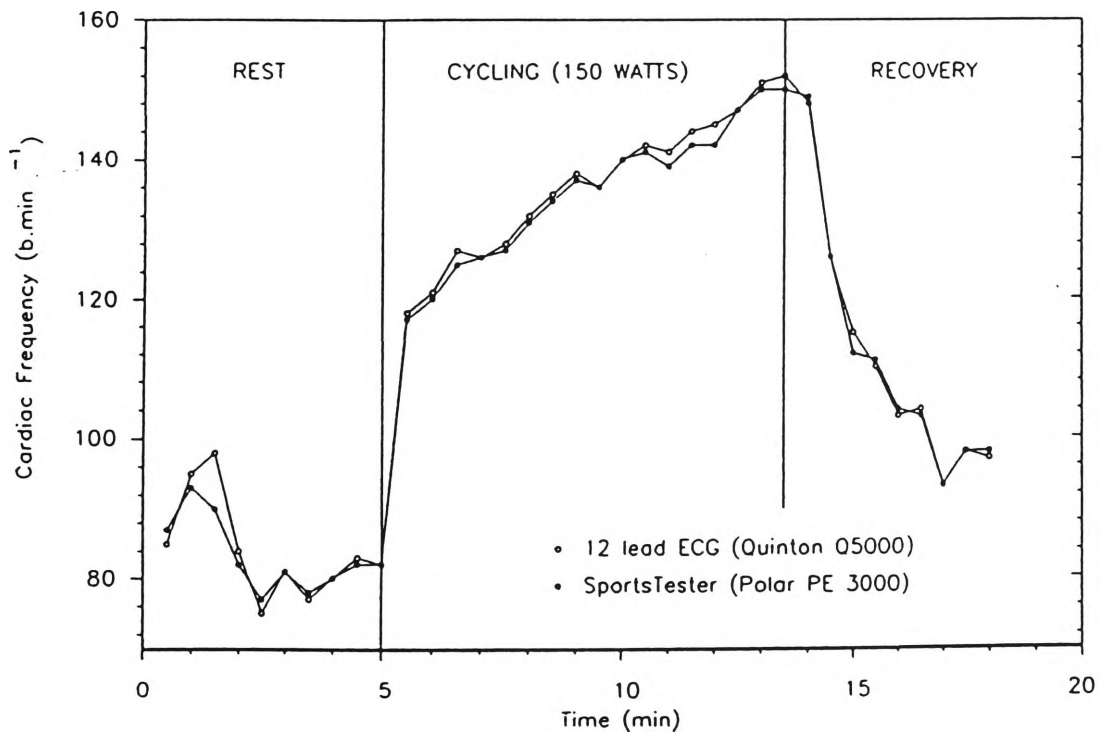
Signature:_____ Date: ___/___/___

Witness: Name_____ Signature:_____

APPENDIX VII

Validation of the Polar PE3000 SportsTester

Accurate monitoring of heart rate was essential in fitness training and testing. The validity and stability of heart rate measurements from the PE 3000 sportstester was compared with those obtained from ECG recordings of the Quinton Qplex 5000 at various heart rate levels. An electronically braked cycle ergometer was used as the exercise stimulus in the validation of the sportstester. The heart rates of two subjects were recorded for four minutes at rest, and again during a PWC 170 cycle ergometer test at three differing workloads. The workloads were 100 Watts, 150 Watts and 200 Watts. Finally heart rate was recorded for four minutes during recovery following the completion of the PWC 170. The collection of beats recorded from both devices over this period were plotted in order to obtain correlation coefficients.



APPENDIX VIII

Calibration of the Quinton Treadmill

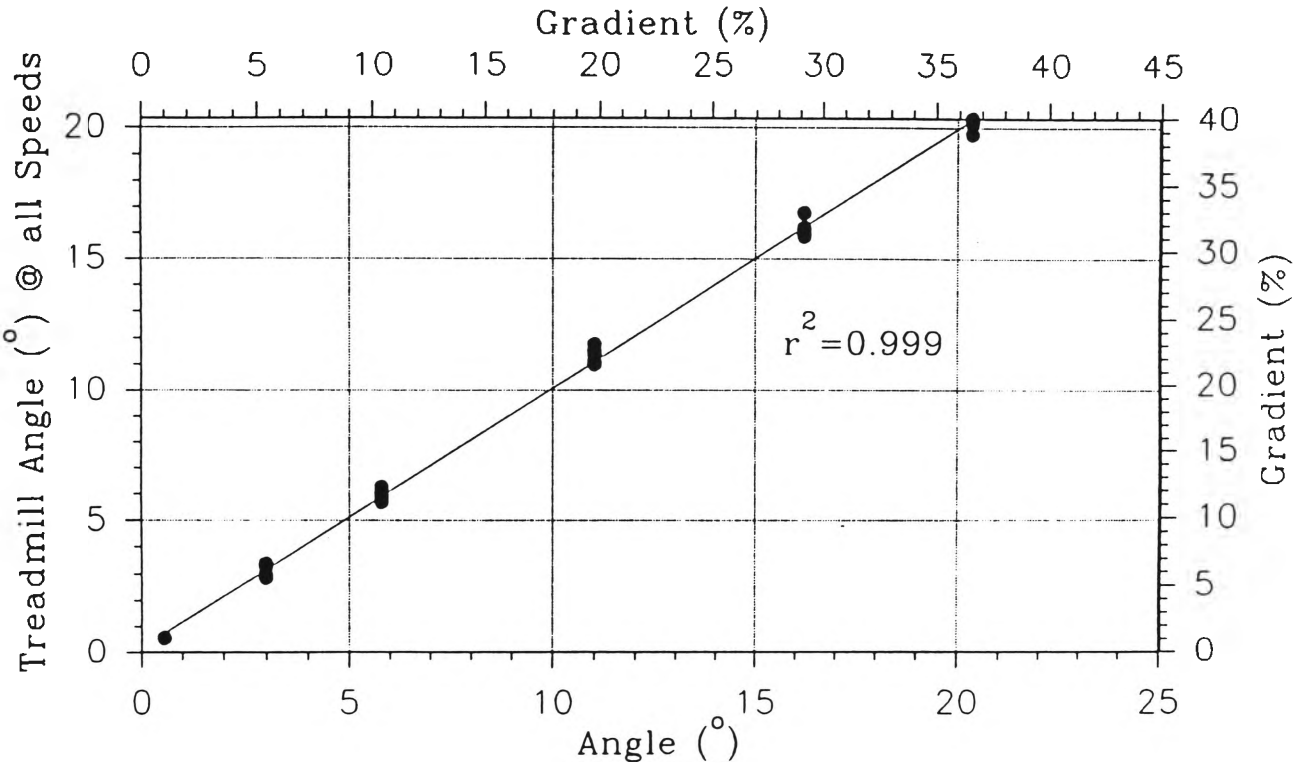
Elevation Calibration

The incline of the treadmill is expressed in units called "per-cent grade", which is defined as the amount of vertical rise per 100 units of belt travel. Grades of 0%, 5%, 10%, 20%, 30% and 40% were calibrated while loaded and unloaded. Grade was also converted to angle in degrees using the formula (degrees = 1/percent grade INV tangent). A carpenters level was first used to check the zero grade on the meter. The treadmill was then elevated through each of the aforementioned grades and the carpenters tool used to measure grade at each level unloaded and loaded with a weight of approximately 75 kilograms. The resulting values were then graphed.

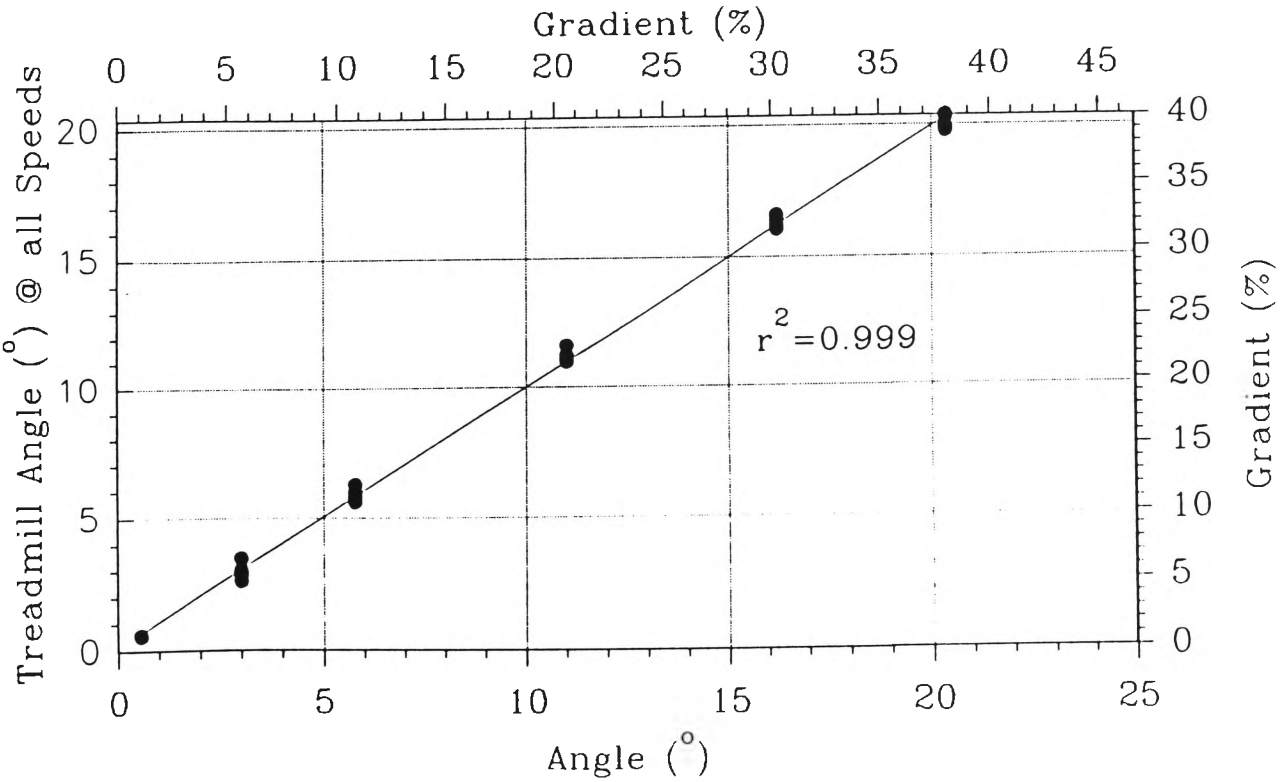
Speed Calibration

The length of the treadmill belt was measured at 439.2cm following which a small piece of tape was placed on the belt surface near the edge. The treadmill was then turned on to a given speed. The speeds measured were 5kph, 7.5kph, 10kph, 15kph, 20kph and 24kph. These speeds were calibrated through each of the previously mentioned grades. The time in seconds was recorded as the belt passed through 15 revolutions. The number of revolutions was converted to revolutions per minute. This was then multiplied by belt length to obtain metres/minute, then divided by 26.8 to convert to miles/hour and finally multiplied by 1.6 to kilometres/hour. The procedure was repeated while a subject with a weight of approximately 75 kilograms ran on the belt. The results were then graphed.

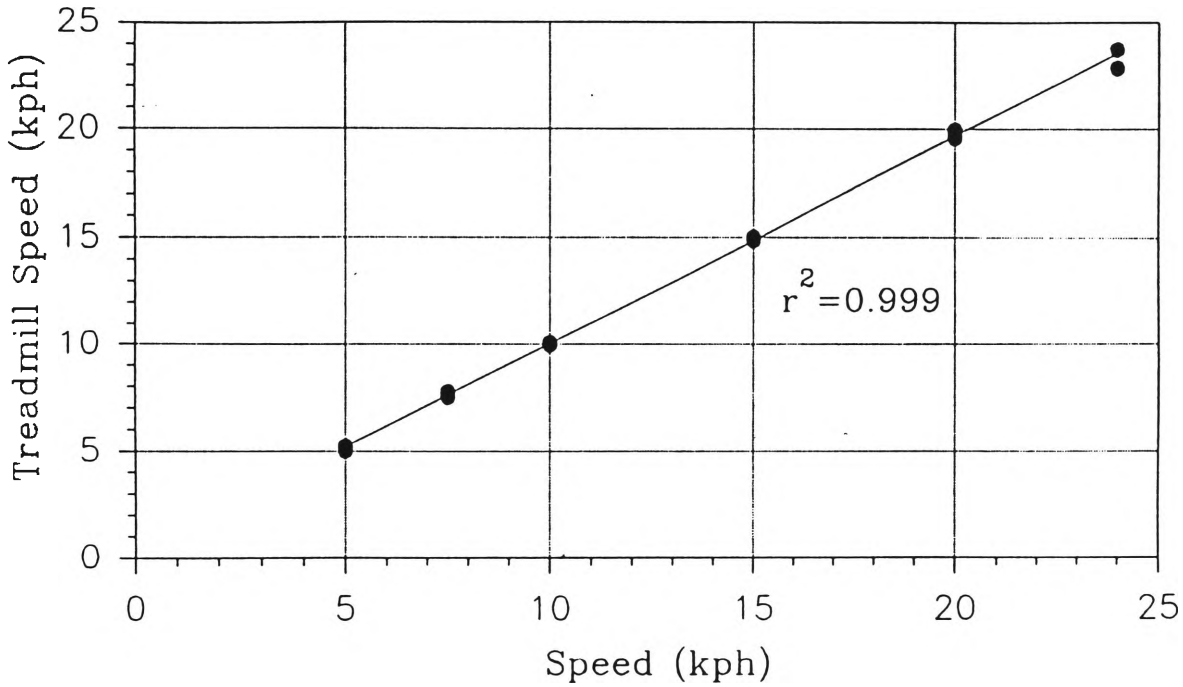
Calibration of treadmill grade at all speeds (Loaded).



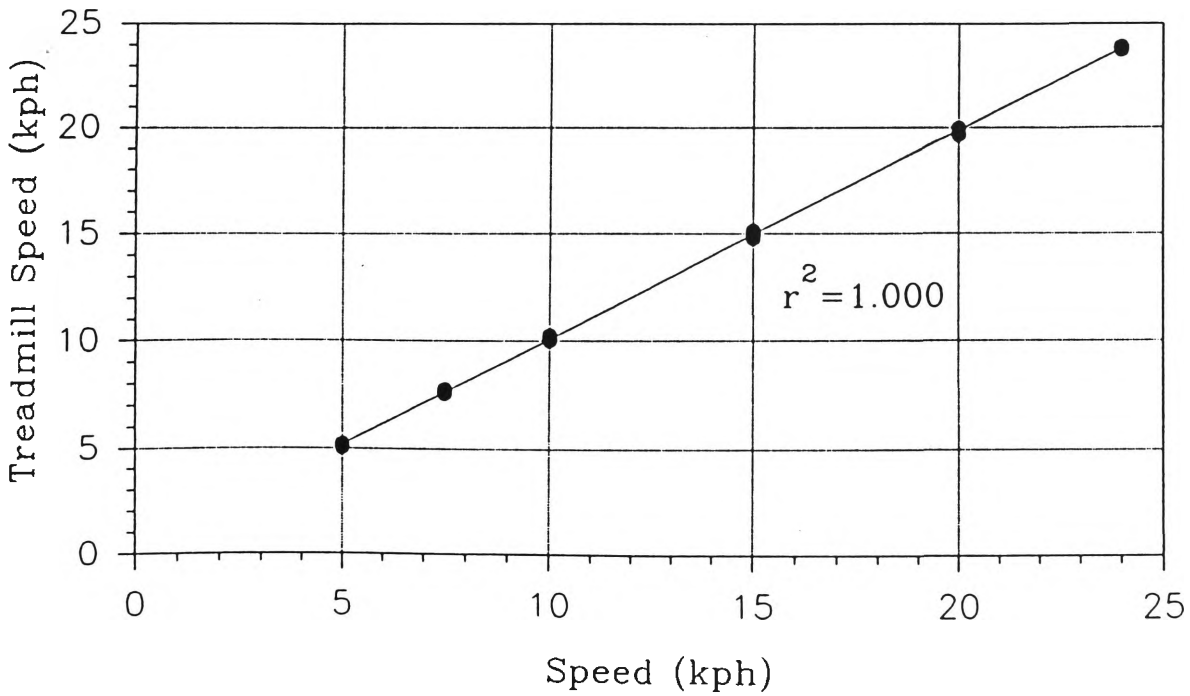
Calibration of treadmill grade at all speeds (Unloaded).



Calibration of treadmill speed at all grades (Loaded).



Calibration of treadmill speed at all grades (Unloaded).



APPENDIX IX

Automatic Calibration of the Norland XR-26 Bone Densitometer

To begin the calibration process *Scanner* command is selected and click on *Begin Calibration* command. You are then asked to place the calibration standard in its position on the scanner table. The bracket on the calibration standard incorporates keyhole-shaped openings to allow the standard to be locked in position. The scanner arm should be positioned near the foot of the table. The laser spot is moved as close as possible to spot "A" marked on the top of the standard and then marked. The same is done for spot "B" which is marked on top of the standard. When point "B" is marked the XR-26 turns the laser off and completes the calibration. It completes a series of instrument diagnostics indicating pass or fail status for reference beam, shutter, high, medium and low attenuation filter tests. If an error is detected, a message explaining the that the calibration is not able to be completed appears on screen. After the quality control diagnostics have been successfully completed the calibration standard is scanned. The scanner arm moves to pre-programmed positions and measurements are made following which calibration results are displayed and printed when the process is complete. The results are saved in disk memory.

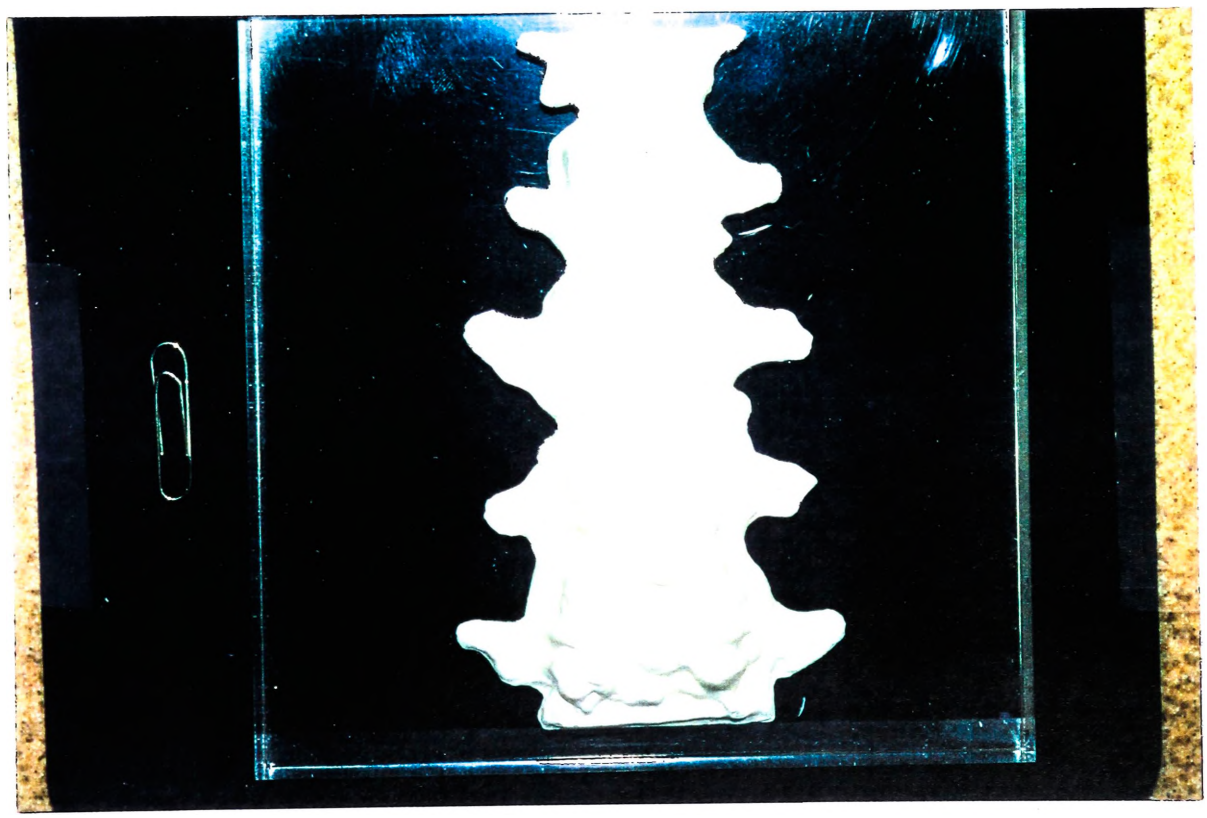
APPENDIX X

Equation used to calculate the coefficient of variation of the Norland XR-26 bone densitometer.

$$CV = \frac{\text{standard deviation} \times 100}{\text{mean}}$$

$$BMD = CV = \frac{0.005 \times 100}{0.857}$$
$$= 0.58\%$$

$$BMC = CV = \frac{0.454 \times 100}{34.19}$$
$$= 1.33\%$$



APPENDIX XI

Instructions to subjects for the fasting 2 hour urine for hydroxyproline

1. **6pm: The night before the test** - Nothing more to eat or drink until instructed.
2. **7.30-8am: Morning of the test** - Drink 2 large glasses of water - approximately 180 ml each. Nothing else to eat or drink.
3. **8am:** - Pass urine into toilet to completely empty bladder. This is the beginning of the test. **Write down the exact time** on the bottle label. From this time onwards, all urine is passed into the container provided.
4. **8.30am:** - Drink 2 more 180ml glasses of water.
5. **10am:** - Pass urine into container for the last time. This completes the test. **Write down the exact time** on the bottle label.
6. Keep in the refrigerator (NOT freezer) until collected.

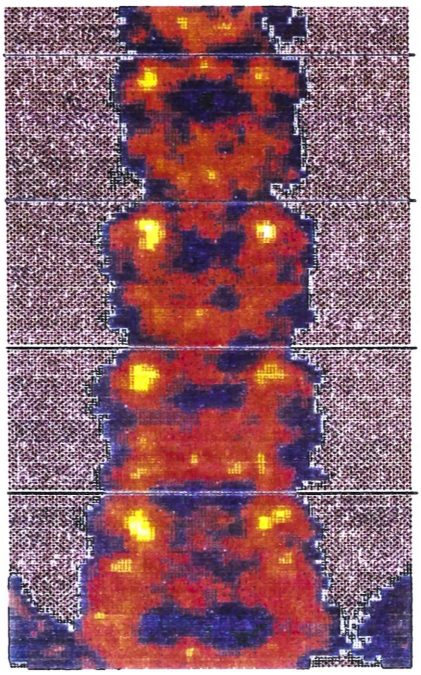
APPENDIX XII

DEXA scan of the lumbar spine.

ILLAWARRA NUCLEAR IMAGING BONE DENSITY ASSESSMENT

Image not for diagnostic purposes.

L H



Tel.: Male Menoage:
Height: Weight: Age:
Arm Span: Ethnic:
Bone History:

Treatment:

Other Medications:

Comments:

Technician:
Physician:

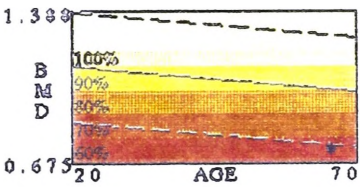
60.0mm/sec 1.5x1.5mm 10.05cm NORLAND Rev:2.2.2/1.1.5 Cal:25/09/91

AP Spine 1 25/09/91

	BMD g/cm²	BMC g	AREA cm²	LENGTH cm
L2	0.701 (2.0)	10.93 (2.0)	15.60 (1.0)	3.60 (ref)
L3	0.742 (2.0)	13.02 (2.0)	17.54 (1.0)	3.60 (ref)
L4	0.757 (2.0)	14.31 (2.0)	18.91 (1.0)	3.60 (ref)
L2-L4	0.735 (1.0)	38.26 (1.5)	52.04 (1.0)	10.80 (ref)

(CVs shown in percent)

L2 - L4 CAUCASIAN WESTERN EUROPE



% Young Ref. 65.4
% Age Matched 72.2
Z - Score -2.2

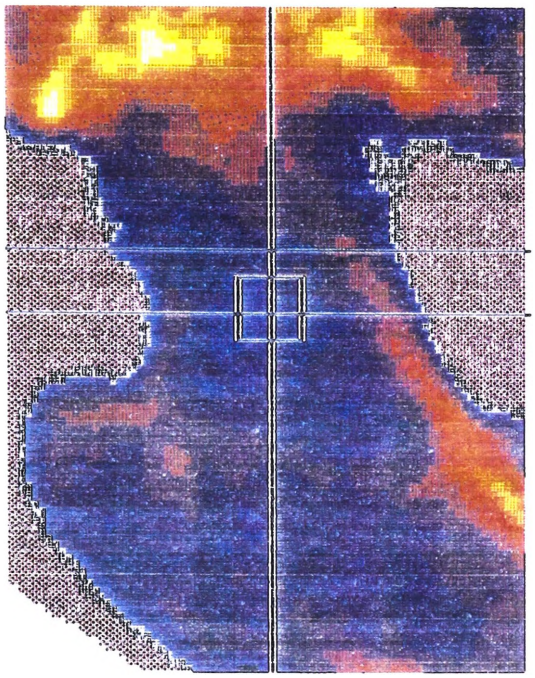
APPENDIX XII (cont)

DEXA scan of the proximal femur.

ILLAWARRA NUCLEAR IMAGING BONE DENSITY ASSESSMENT

Image not for diagnostic purposes.

L H



Tel.: Male Menoage:
Height: Weight: Age:
Arm Span: Ethnic:
Bone History:

Treatment:

Other Medications:

Comments:

Technician:
Physician:

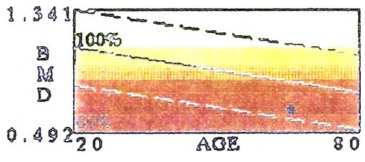
45.0mm/sec 1.0x1.0mm 8.00cm NORLAND Rev:2.2.2/1.1.5 Col:25/09/91

Right Hip 2 25/09/91

Fem Neck CAUCASIAN
COMBINED

	BMD g/cm²	BMC g	AREA cm²	LENGTH cm
NECK	0.640 (2.5)	2.74 (2.0)	4.28 (1.5)	1.00 (ref)
TROCH.	0.651 (2.0)	10.57 (4.5)	16.25 (4.5)	
WARD'S	0.458 (4.5)	0.46 (4.5)	1.00 (ref)	

(CVs shown in percent)



% Young Ref. 59.8
% Age Matched 76.5
Z - Score -1.5

APPENDIX XIII

The exercise and non-exercise groups training diaries

Subject	No. supervised runs /46(%)	No. supervised runs (min)	Unsupervised runs (min)	Additional exercise
R1	27 (58.7)	870	365	1065
R2	16 (34.8)	435	440	550
R3	15 (32.6)	425	430	8287
R4	18 (39.1)	495	480	4890
R5	39 (84.7)	1210	0	510
R6	38 (82.6)	1140	295	4490
R7	26 (56.5)	765	0	0
R8	23 (50.0)	675	570	5070
R9	10 (21.7)	210	885	7210
R10	15 (32.6)	395	1035	6500
R11	23 (50.0)	680	560	3680
R12	19 (41.3)	495	480	10050
Mean	**22 (47.8)**	**649**	**461**	**4358.5**

Subject	Additional exercise (min)	Type of activity
C1	645	rollerblading
C2	300	walking
C3	480	walking
C4	3330	soccer, gym, netball, jogging, swimming, badminton
C5	1030	cycling, jogging, bushwalking, tennis, basketball
C6	360	cycling
C7	1380	jogging, swimming
C8	1200	volleyball, basketball
C9	2100	gym, jogging
C10	1080	walking
MEAN	**1188 = 74 min/week**	

APPENDIX XIV

Raw data (Pre and Post test)- means and SD for anthropometric data of both the exercise (R) and non-exercise (C) groups.

Subject Number	Age	Height	Weight	
			Pre test	Post test
R1	23.0	178.8	81.52	70.60
R2	20.8	180.3	69.10	68.08
R3	20.4	176.2	62.10	62.03
R4	22.5	190.6	91.32	89.94
R5	25.9	185.3	84.10	83.06
R6	20.0	177.9	74.00	71.98
R7	27.1	168.6	72.00	72.12
R8	22.4	175.9	87.00	81.78
R9	20.0	173.0	63.54	63.10
R10	21.2	179.1	72.44	75.43
R11	24.9	170.6	58.12	58.10
R12	22.8	183.9	80.82	79.00
Mean	22.6	178.4	74.59	72.94
SD	2.3	6.2	10.51	9.44

Subject Number	Age	Height	Weight	
			Pre test	Post test
C1	20.3	178.2	76.12	77.21
C2	20.9	182.1	84.79	88.38
C3	20.4	177.6	84.26	84.06
C4	23.8	181.3	83.34	86.20
C5	23.5	189.4	79.84	81.50
C6	23.4	174.8	72.92	71.84
C7	20.0	178.5	80.40	77.78
C8	20.2	172.5	66.62	67.78
C9	24.8	178.2	80.12	80.66
C10	20.3	176.6	71.52	68.30
Mean	21.8	178.9	77.99	78.37
SD	1.9	4.4	6.04	7.20

APPENDIX XV

Raw data (pre test)- means, SD and SEM of the exercise group (R) and non-exercise group (C) for bone mineral density at all sites.

Subject No.	Femoral Neck	Troch.	Ward's Tri.	L2-L4	L2	L3	L4
R1	1.035	0.974	1.032	1.197	1.173	1.233	1.184
R2	1.161	1.159	1.210	1.297	1.322	1.288	1.282
R3	0.888	0.847	0.824	0.921	0.926	0.956	0.884
R4	1.358	1.142	1.360	1.211	1.270	1.232	1.136
R5	1.137	0.942	1.146	1.393	1.488	1.294	1.406
R6	1.084	0.914	1.147	0.946	0.926	0.984	0.929
R7	0.700	0.703	0.710	0.845	0.808	0.873	0.847
R8	0.846	0.833	0.964	1.072	1.085	1.098	1.037
R9	0.903	0.761	0.836	0.989	1.011	0.953	0.999
R10	1.062	0.870	1.020	1.116	1.111	1.131	1.105
R11	1.180	1.069	0.940	1.325	1.381	1.331	1.298
R12	1.171	1.072	1.202	1.058	1.112	1.127	0.957
Mean	1.035	0.941	1.033	1.114	1.135	1.125	1.089
SD	0.176	0.147	0.189	0.173	0.203	0.155	0.178
SEM	0.051	0.043	0.055	0.050	0.059	0.045	0.051

Subject No.	Femoral Neck	Troch.	Ward's Tri.	L2-L4	L2	L3	L4
C1	1.070	0.894	1.310	1.014	1.011	1.016	1.015
C2	0.950	0.815	1.121	0.919	0.906	0.958	0.902
C3	1.182	0.901	1.179	1.274	1.294	1.285	1.249
C4	1.357	1.067	1.451	1.243	1.249	1.262	1.223
C5	1.042	0.806	0.915	1.045	1.003	1.079	1.051
C6	1.090	0.854	1.096	0.965	0.966	0.989	0.943
C7	0.889	0.813	0.857	0.946	0.923	0.965	0.949
C8	1.076	0.909	1.176	0.925	0.905	0.940	0.928
C9	1.073	0.923	1.012	1.068	1.092	1.068	1.048
C10	1.150	0.839	1.110	0.932	0.953	0.953	0.891
Mean	1.088	0.882	1.123	1.033	1.030	1.052	1.020
SD	0.128	0.078	0.175	0.130	0.140	0.126	0.127
SEM	0.040	0.025	0.055	0.041	0.044	0.040	0.040

APPENDIX XVI

Raw data (post test)- means, SD, and SEM of the exercise group (R) and non-exercise group (C) for bone mineral density at all sites.

Subject No.	Femoral Neck	Troch.	Ward's Tri.	L2-L4	L2	L3	L4
R1	1.030	0.966	1.028	1.130	1.105	1.183	1.101
R2	1.165	1.098	1.164	1.271	1.248	1.295	1.270
R3	0.883	0.804	0.800	0.933	0.917	0.980	0.903
R4	1.359	1.151	1.352	1.189	1.265	1.210	1.097
R5	1.130	0.918	1.137	1.398	1.390	1.447	1.353
R6	1.082	0.937	1.154	0.969	0.968	0.973	0.966
R7	0.805	0.755	0.750	0.880	0.922	0.881	0.844
R8	0.812	0.786	0.968	1.033	1.089	1.080	0.936
R9	0.902	0.768	0.819	0.982	0.998	0.966	0.983
R10	1.041	0.770	0.904	1.124	1.046	1.157	1.167
R11	1.126	1.181	1.001	1.320	1.355	1.320	1.306
R12	1.244	1.008	1.266	1.071	1.147	1.126	0.966
MEAN	1.048	0.929	1.029	1.108	1.121	1.125	1.074
SD	0.172	0.156	0.191	0.162	0.163	0.168	0.169
SEM	0.050	0.045	0.055	0.047	0.047	0.049	0.049

Subject No.	Femoral Neck	Troch.	Ward's Tri.	L2-L4	L2	L3	L4
C1	1.152	0.889	1.171	1.047	1.019	1.035	1.083
C2	0.981	0.817	1.136	0.929	0.912	0.958	0.915
C3	1.132	1.007	1.150	1.251	1.275	1.241	1.239
C4	1.313	1.052	1.345	1.232	1.227	1.243	1.226
C5	1.040	0.832	1.013	1.056	0.996	1.123	1.047
C6	1.084	0.854	1.082	0.935	0.905	0.969	0.931
C7	0.902	0.809	0.861	1.035	0.994	1.064	1.043
C8	1.090	0.932	1.172	0.938	0.910	0.949	0.951
C9	1.096	0.939	1.050	1.110	1.091	1.139	1.101
C10	1.134	0.846	1.052	0.898	1.134	0.846	1.052
Mean	1.092	0.898	1.103	1.043	1.046	1.057	1.059
SD	0.109	0.083	0.126	0.125	0.132	0.131	0.112
SEM	0.035	0.026	0.040	0.040	0.042	0.041	0.035

APPENDIX XVII

Raw data (pre test)- means, SD and SEM of the exercise (R) and non-exercise (C) groups for bone mineral content at all sites.

Subject No.	Femoral Neck	Troch.	Ward's Tri.	L2-L4	L2	L3	L4
R1	3.04	14.17	1.03	58.85	16.46	17.95	18.43
R2	4.26	15.71	1.21	57.98	19.38	18.40	20.20
R3	3.23	10.42	0.82	45.41	13.80	15.71	15.91
R4	5.36	19.51	1.36	66.30	22.69	21.93	21.69
R5	4.96	14.80	1.15	60.38	19.58	19.14	21.65
R6	3.74	13.40	1.15	39.94	12.35	13.35	14.24
R7	2.83	8.43	0.72	34.50	9.72	12.71	12.06
R8	3.45	13.66	0.96	46.73	15.06	15.18	16.49
R9	3.11	10.03	0.84	38.25	12.23	11.92	14.11
R10	4.45	12.12	1.03	48.93	15.06	16.48	17.39
R11	3.94	11.59	0.94	59.29	19.58	19.51	20.19
R12	5.26	16.96	1.36	49.47	16.62	15.92	16.93
Mean	3.97	13.40	1.05	50.00	16.04	16.52	17.44
SD	0.89	3.13	0.21	9.74	3.77	3.00	3.10
SEM	0.26	0.90	0.06	2.81	1.09	0.87	0.90

Subject No.	Femoral Neck	Troch.	Ward's Tri.	L2-L4	L2	L3	L4
C1	4.46	13.19	0.96	41.71	13.12	13.55	15.05
C2	4.11	9.87	1.12	46.71	14.05	16.70	15.95
C3	4.29	14.39	1.18	57.22	1.70	19.75	20.40
C4	5.29	14.59	1.45	57.57	18.55	18.16	20.86
C5	4.57	9.91	0.92	55.56	16.76	19.16	19.58
C6	4.31	11.58	1.10	45.47	14.05	15.34	16.08
C7	3.81	12.49	0.86	38.65	11.94	13.71	13.00
C8	3.71	13.32	1.10	41.82	12.19	14.66	14.98
C9	4.29	13.38	1.01	52.42	16.43	16.94	19.04
C10	3.84	13.13	1.11	44.81	14.53	15.73	14.55
Mean	4.27	12.59	1.08	48.19	14.87	16.37	16.95
SD	0.46	1.66	0.16	6.96	2.23	2.17	2.77
SEM	0.15	0.52	0.05	2.20	0.70	0.69	0.88

APPENDIX XVIII

Raw data (Post test)- means, SD, and SEM of the exercise (R) and non-exercise (C) groups for bone mineral content at all sites.

Subject No.	Femoral Neck	Troch.	Ward's Tri.	L2-L4	L2	L3	L4
R1	3.76	15.40	1.03	49.77	15.10	17.48	17.20
R2	4.41	15.04	1.16	54.72	17.56	17.61	19.55
R3	3.13	16.77	0.80	46.31	13.67	16.28	16.36
R4	5.35	19.07	1.35	63.20	22.02	21.04	20.13
R5	4.72	11.80	1.14	58.91	17.50	22.09	19.32
R6	3.78	6.92	1.15	41.16	12.97	13.22	14.97
R7	2.96	4.02	0.75	36.67	11.47	13.07	12.14
R8	3.44	13.64	0.97	44.86	15.85	14.96	14.05
R9	3.21	6.66	0.82	37.69	11.94	12.07	18.68
R10	4.33	9.05	0.90	49.84	15.28	16.61	17.95
R11	4.03	12.94	1.00	57.77	11.89	19.45	19.43
R12	4.07	15.05	1.27	48.93	16.13	16.03	16.77
Mean	3.93	12.20	1.03	49.15	15.75	16.67	16.80
SD	0.70	4.60	0.19	8.40	3.09	3.12	2.63
SEM	0.20	1.33	0.06	2.43	0.89	0.90	0.76

Subject No.	Femoral Neck	Troch.	Ward's Tri.	L2-L4	L2	L3	L4
C1	4.45	4.68	1.17	43.46	13.30	13.86	16.40
C2	4.07	10.00	1.14	47.56	14.35	16.93	16.29
C3	4.30	12.17	1.15	56.16	17.43	18.68	20.04
C4	4.97	11.77	1.34	56.58	17.86	17.68	21.04
C5	4.33	12.26	1.01	56.51	16.81	19.84	19.85
C6	4.40	11.33	1.08	42.94	13.07	14.04	15.83
C7	3.71	13.08	0.86	43.28	13.03	15.60	14.64
C8	3.69	15.46	1.17	42.71	12.64	14.92	15.15
C9	4.18	14.40	1.05	53.78	16.73	17.27	19.78
C10	3.86	12.40	1.03	42.09	13.85	14.67	13.57
Mean	4.20	11.76	1.10	48.41	14.91	16.35	17.26
SD	0.39	2.91	0.13	6.55	2.06	2.04	2.66
SEM	0.12	0.92	0.04	2.07	0.65	0.65	0.84

APPENDIX XIX

Raw data (Pre and Post test)- means, SD, and SEM for the exercise and non-exercise groups for the bone markers serum osteocalcin and urinary hydroxyproline.

Subject Number	Serum Osteocalcin		Urinary Hydroxyproline	
	Pre test	Post test	Pre test	Post test
R1	6.9	7.5	14.6	22.1
R2	22.0	14.8	35.2	20.0
R3	22.3	10.9	20.0	30.8
R4	11.4	12.8	13.3	17.4
R5	8.3	*	9.5	12.9
R6	13.1	16.1	30.0	25.1
R7	8.5	10.6	8.6	12.1
R8	10.3	11.3	11.9	18.4
R9	4.9	17.7	14.3	16.7
R10	17.1	8.0	24.6	14.7
R11	8.8	9.0	8.3	7.1
R12	12.0	17.4	16.4	25.1
Mean	12.1	12.4	17.2	18.5
SD	5.6	5.6	8.6	6.6
SEM	1.6	1.1	2.5	1.9

Subject Number	Serum Osteocalcin		Urinary Hydroxyproline	
	Pre test	Post test	Pre test	Post test
C1	28.6	21.4	19.7	14.0
C2	13.7	12.3	15.1	16.8
C3	7.9	13.3	16.8	20.1
C4	22.7	11.7	17.9	16.5
C5	4.0	8.6	15.0	22.8
C6	22.3	19.6	14.5	11.9
C7	9.0	14.2	24.2	12.9
C8	12.3	12.4	34.0	16.5
C9	23.1	6.1	20.9	12.0
C10	11.7	13.1	22.8	21.9
Mean	15.5	13.3	20.1	16.5
SD	8.1	4.5	5.9	4.0
SEM	2.6	1.4	1.9	1.3

APPENDIX XX

Raw data (Pre and Post test)- means, SD, and SEM for cardiovascular fitness of the exercise (R) and non-exercise (C) groups.

Subject Number	Cumulative heart rate (beats)		% change	Time to Exhaustion (min)	
	Pre test	Post test		Pre test	Post test
R1	1682	1354	-19.5	8	10
R2	1875	1753	-6.5	11	12
R3	1928	1857	-3.7	11	12
R4	1621	1553	-4.2	10	11
R5	1899	1904	0.3	11	12
R6	2093	2050	-2.1	12	13
R7	1429	1287	-9.9	7	11
R8	1593	1426	-10.5	9	11
R9	2089	2056	-1.6	12	13
R10	2126	2124	-0.1	12	12
R11	1941	1784	-8.1	11	12
R12	2066	1910	-7.6	12	13
Mean	1861.83	1754.83	-6.13	10.5	11.8
SD	229.36	286.06	5.58	1.7	0.9
SEM	66.21	82.58	1.61	0.5	0.3

Subject Number	Cumulative heart rate (beats)		% change	Time to Exhaustion (min)	
	Pre test	Post test		Pre test	Post test
C1	1998	1944	-2.7	12	12
C2	1905	1888	-0.9	11	11
C3	1293	1444	11.7	7	7
C4	1411	1505	6.7	10	9
C5	1937	1977	2.1	11	11
C6	1931	1875	-2.9	10	10
C7	1297	1189	-8.3	8	7
C8	1558	1552	-0.4	11	9
C9	1697	1650	-2.8	10	10
C10	1943	1960	-0.9	12	11
Mean	1697.00	1698.40	0.16	10.2	9.7
SD	285.01	270.57	5.58	1.6	1.7
SEM	90.12	85.56	1.76	0.5	0.5

APPENDIX XXI

Maximal heart rates achieved from the maximal exercise treadmill test for both exercise and control subjects.

Maximal Heart Rates						
Exercise Group R1-R12					Control Group C1-C10	
Pre Test 1	Test 2	Test 3	Test 4	Post test 5	Pre Test 1	Post test 2
209	203	196	198	196	181	177
192	199	201	*	196	200	199
185	180	188	195	194	190	205
186	185	179	180	190	191	198
191	195	197	186	196	204	209
191	198	190	193	196	197	194
207	198	*	207	214	188	161
184	186	180	*	187	187	181
194	189	197	*	199	196	189
200	196	203	*	201	192	199
186	186	182	188	187		
189	186	189	*	189		

* Denotes absent from Maximal exercise test.

APPENDIX XXII

Correlations between subjects following re-positioning for bone mineral density and bone mineral content at all sites.

BMD(g/cm^2) Subjects	Femoral Neck		Greater Trochanter		Ward's Triangle	
	Scan 1	Scan 2	Scan 1	Scan 2	Scan 1	Scan 2
1	1.182	1.195	0.981	1.006	1.179	1.215
2	1.184	1.181	0.914	0.931	1.147	1.209
3	0.903	0.940	0.761	0.755	0.836	0.837
4	1.150	1.164	0.839	0.842	1.110	1.221
5	1.090	1.115	0.854	0.877	1.096	1.129
r	0.996		0.993		0.958	

BMC(g/cm^2) Subjects	Femoral Neck		Greater Trochanter		Ward's Triangle	
	Scan 1	Scan 2	Scan 1	Scan 2	Scan 1	Scan 2
1	4.29	4.32	14.39	14.85	1.18	1.22
2	3.74	3.77	13.40	12.23	1.15	1.21
3	3.11	3.20	10.03	11.37	0.84	0.84
4	3.84	3.94	13.13	12.44	1.11	1.22
5	4.31	4.36	11.58	12.93	1.10	1.13
r	0.997		0.545		0.959	

BMD(g/cm^2) Subjects	L2		L3		L4		L2-L4	
	Scan 1	Scan 2	Scan 1	Scan 2	Scan 1	Scan 2	Scan 1	Scan 2
1	1.286	1.199	1.209	1.195	1.208	1.170	1.231	1.187
2	1.043	1.106	1.089	1.085	1.219	1.180	1.125	1.128
3	1.325	1.263	1.303	1.317	1.273	1.241	1.298	1.272
4	1.066	1.040	1.146	1.101	1.053	1.056	1.089	1.067
5	1.318	1.339	1.359	1.434	1.302	1.334	1.325	1.363
r	0.816		0.966		0.906		0.932	

BMD(g/cm^2) Subjects	L2		L3		L4		L2-L4	
	Scan 1	Scan 2	Scan 1	Scan 2	Scan 1	Scan 2	Scan 1	Scan 2
1	18.18	16.66	19.40	18.98	21.37	20.51	58.95	56.14
2	12.87	13.28	15.46	14.82	19.82	18.67	48.15	46.78
3	15.42	14.95	17.58	17.48	19.76	19.60	52.76	52.03
4	12.68	12.41	16.50	15.79	16.33	16.13	45.51	47.33
5	22.64	22.95	20.06	21.39	23.14	23.75	65.84	68.09
r	0.966		0.953		0.944		0.939	

Appendix XXIII

Student t-tests tables for all pre-test data.

Variable: BMC Femoral neck

Variances	t-value	df	2-tail sig
Equal	0.96	20	0.311

Variable: BMC Greater Trochanter

Variances	t-value	df	2-tail sig
Equal	0.74	20	0.468

Variable: BMC Ward's triangle

Variances	t-value	df	2-tail sig
Equal	0.42	20	0.081

Variable: BMC L2-L4

Variances	t-value	df	2-tail sig
Equal	0.49	20	0.629

Variable: BMC L2

Variances	t-value	df	2-tail sig
Equal	0.87	20	0.396

Variable: BMC L3

Variances	t-value	df	2-tail sig
Equal	0.13	20	0.899

Variable: BMC L4

Variances	t-value	df	2-tail sig
Equal	0.39	20	0.702

Variable: BMD Femoral neck

Variances	t-value	df	2-tail sig
Equal	0.78	20	0.442

Variable: BMD Greater Trochanter

Variances	t-value	df	2-tail sig
Equal	1.13	20	0.273

APPENDIX XXIII (cont)

Variable: BMD Ward's triangle

Variances	t-value	df	2-tail sig
Equal	1.15	20	0.263

Variable: BMD L2-L4

Variances	t-value	df	2-tail sig
Equal	1.22	20	0.237

Variable: BMD L2

Variances	t-value	df	2-tail sig
Equal	1.38	20	0.076

Variable: BMD L3

Variances	t-value	df	2-tail sig
Equal	1.20	20	0.243

Variable: BMD L4

Variances	t-value	df	2-tail sig
Equal	1.02	20	0.318

Variable: Serum Osteocalcin

Variances	t-value	df	2-tail sig
Equal	1.16	20	0.260

Variable: Urinary Hydroxyproline

Variances	t-value	df	2-tail sig
Equal	0.89	20	0.386

Variable: Area under curve (cumulative heart rate)

Variances	t-value	df	2-tail sig
Equal	1.50	20	0.148

Variable: Time to exhaustion

Variances	t-value	df	2-tail sig
Equal	0.42	20	0.707

Variable: Weight

Variances	t-value	df	2-tail sig
Equal	0.91	20	0.376

APPENDIX XXIII (cont)

Variable: Height

Variances	t-value	df	2-tail sig
Equal	0.56	20	0.584

Variable: Age

Variances	t-value	df	2-tail sig
Equal	1.00	20	0.331

APPENDIX XXIV

Student t-test tables for the difference between the means of all the data.

Variable: BMD Femoral neck

	No. of cases	mean	SD	SEM
exercise	12	0.0128	0.041	0.012
control	10	0.0045	0.038	0.012

Variances	t-value	df	2-tail sig
Pooled	0.49	20	0.628

Variable: BMD Greater Trochanter

	No. of cases	mean	SD	SEM
exercise	12	-0.0120	0.058	0.017
control	10	0.0156	0.034	0.011

Variance	t-value	df	2-tail sig
Pooled	1.33	20	0.200

Variable: BMD Ward's triangle

	No. of cases	mean	SD	SEM
exercise	12	-0.0040	0.049	0.014
control	10	-0.0195	0.069	0.022

Variance	t-value	df	2-tail sig
Pooled	0.62	20	0.543

Variable: BMD L2-L4

	No. of cases	mean	SD	SEM
exercise	12	-0.0058	0.029	0.008
control	10	0.0100	0.038	0.012

Variance	t-value	df	2-tail sig
Pooled	1.12	20	0.277

Variable: BMD L2

	No. of cases	mean	SD	SEM
exercise	12	0.0139	0.059	0.017
control	10	0.0161	0.067	0.021

Variance	t-value	df	2-tail sig
Pooled	1.12	20	0.277

APPENDIX XXIV (cont)

Variable: BMD L3

	No. of cases	mean	SD	SEM
exercise	12	0.0098	0.050	0.014
control	10	0.0052	0.059	0.019

Variance	t-value	df	2-tail sig
Pooled	0.20	20	0.843

Variable: BMD L4

	No. of cases	mean	SD	SEM
exercise	12	-0.0143	0.048	0.014
control	10	0.0389	0.056	0.018

Variance	t-value	df	2-tail sig
Pooled	2.41	20	-0.026

Variable: BMC Femoral neck

	No. of cases	mean	SD	SEM
exercise	12	-0.0367	0.433	0.125
control	10	-0.0720	0.125	0.040

Variance	t-value	df	2-tail sig
Pooled	0.25	20	0.806

Variable: BMC Greater Trochanter

	No. of cases	mean	SD	SEM
exercise	12	-1.2033	3.327	0.961
control	10	-0.8300	3.171	1.003

Variance	t-value	df	2-tail sig
Pooled	0.27	20	0.792

Variable: BMC Ward's Triangle

	No. of cases	mean	SD	SEM
exercise	12	-0.0192	0.051	0.015
control	10	0.0210	0.089	0.028

Variance	t-value	df	2-tail sig
Pooled	1.32	20	0.200

APPENDIX XXIV (cont)

Variable: BMC L2-L4

	No. of cases	mean	SD	SEM
exercise	12	-0.8500	1.845	0.533
control	10	0.2130	1.999	0.632

Variance	t-value	df	2-tail sig
Pooled	1.30	20	0.210

Variable: BMC L2

	No. of cases	mean	SD	SEM
exercise	12	-.2992	1.101	0.318
control	10	0.0380	0.634	0.201

Variance	t-value	df	2-tail sig
Pooled	0.86	20	0.403

Variable: BMC L3

	No. of cases	mean	SD	SEM
exercise	12	0.1425	0.985	0.284
control	10	0.3100	0.782	0.247

Variance	t-value	df	2-tail sig
Pooled	0.39	20	0.701

Variable: BMC L4

	No. of cases	mean	SD	SEM
exercise	12	-0.6450	1.070	0.309
control	10	0.3100	0.782	0.247

Variance	t-value	df	2-tail sig
Pooled	2.34	20	0.029

Variable: Serum Osteocalcin

	No. of cases	mean	SD	SEM
exercise	12	-0.1091	6.887	2.076
control	10	-2.2600	7.449	2.355

Variance	t-value	df	2-tail sig
Pooled	0.69	20	0.500

APPENDIX XXIV (cont)

Variable: Urinary Hydroxyproline

	No. of cases	mean	SD	SEM
exercise	12	1.3083	7.811	2.255
control	10	-3.5500	7.482	2.366

Variance	t-value	df	2-tail sig
Pooled	1.48	20	0.154

Variable: Area under curve (cumulative heart rate)

	No. of cases	mean	SD	SEM
exercise	12	-107.00	92.810	26.792
control	10	1.40	77.285	24.440

Variance	t-value	df	2-tail sig
Pooled	2.94	20	0.008

Variable: Time to exhaustion

	No. of cases	mean	SD	SEM
exercise	12	1.33	0.985	0.284
control	10	-0.50	0.707	0.224

Variance	t-value	df	2-tail sig
Pooled	4.92	20	0.000

Variable: % difference in cumulative heart rate

	No. of cases	mean	SD	SEM
exercise	12	0.938	0.056	0.016
control	10	1.003	0.056	0.018

Variance	t-value	df	2-tail sig
Pooled	2.70	20	0.014

Variable: Weight

	No. of cases	mean	SD	SEM
exercise	12	-1.653	3.480	1.005
control	10	0.378	2.202	0.696

Variance	t-value	df	2-tail sig
Pooled	1.60	20	0.126

APPENDIX XXV

Analysis of variance tables for the all data. The experimental group has been divided into two groups above and below the mean found for the percentage difference of cumulative heart rate, while the third group is that of the controls.

Group 1 = > 6.13% change in fitness (n = 6)

Group 2 = < 6.13% change in fitness (n = 6)

Group 3 = control (n = 10)

Variable: BMD Femoral neck

Source	df	SS	MS	F	P
Groups	2	0.0046	0.0023	1.5963	0.2287
Within	19	0.0271	0.0014		
Total	21	0.0317			

Variable: BMD Greater Trochanter

Source	df	SS	MS	F	P
Groups	2	0.0052	0.0026	1.0686	0.3632
Within	19	0.0462	0.0024		
Total	21	0.0514			

Variable: BMD Ward's triangle

Source	df	SS	MS	F	P
Groups	2	0.0081	0.0041	1.2547	0.3077
Within	19	0.0615	0.0032		
Total	21	0.0697			

Variable: BMD L2-L4

Source	df	SS	MS	F	P
Groups	2	0.0023	0.0012	1.0639	0.3648
Within	19	0.0209	0.0011		
Total	21	0.0232			

APPENDIX XXV (cont)

Variable: BMD L2

Source	df	SS	MS	F	P
Groups	2	0.0065	0.0032	0.7987	0.4645
Within	19	0.0771	0.0041		
Total	21	0.0835			

Variable: BMD L3

Source	df	SS	MS	F	P
Groups	2	0.0052	0.0026	0.9343	0.4102
Within	19	0.0533	0.0028		
Total	21	0.0585			

Variable: BMD L4

Source	df	SS	MS	F	P
Groups	2	0.0185	0.0093	3.5180	0.0501
Within	19	0.027	0.001		
Total	21	0.032			

Variable: BMC Femoral neck

Source	df	SS	MS	F	P
Groups	2	0.0108	0.0054	0.0469	0.9543
Within	19	2.1966	0.1156		
Total	21	2.2074			

Variable: BMC Greater Trochanter

Source	df	SS	MS	F	P
Groups	2	3.3549	1.6775	0.1520	0.8600
Within	19	209.7132	11.0375		
Total	21	213.0681			

Variable: BMC Ward's triangle

Source	df	SS	MS	F	P
Groups	2	0.0107	0.0053	1.0295	0.3763
Within	19	0.0985	0.0052		
Total	21	0.1092			

APPENDIX XXV (cont)

Variable: BMC L2-L4

Source	df	SS	MS	F	P
Groups	2	9.1635	4.5817	1.2362	0.3128
Within	19	70.4182	3.7062		
Total	21	79.5817			

Variable: BMC L2

Source	df	SS	MS	F	P
Groups	2	0.7155	0.3577	0.4030	0.6739
Within	19	16.8648	0.8876		
Total	21	17.5803			

Variable: BMC L3

Source	df	SS	MS	F	P
Groups	2	1.3810	0.6905	0.7297	0.4951
Within	19	17.9801	0.9453		
Total	21	19.3611			

Variable: BMC L4

Source	df	SS	MS	F	P
Groups	2	5.5294	2.7647	2.9933	0.0741
Within	19	0.027	0.001		
Total	21	0.032			

Variable: Serum Osteocalcin

Source	df	SS	MS	F	P
Groups	2	27.0157	13.5078	0.2505	0.7811
Within	19	970.8110	53.9339		
Total	21	997.8267			

Variable: Urinary Hydroxyproline

Source	df	SS	MS	F	P
Groups	2	130.0133	65.0067	1.0524	0.3686
Within	19	1173.6667	61.7719		
Total	21	1303.6800			

APPENDIX XXV (cont)

Variable: Weight

Source	df	SS	MS	F	P
Groups	2	43.6277	21.8139	2.6608	0.0958
Within	19	155.7637	8.1981		
Total	21	199.3914			

APPENDIX XXVI

Correlation coefficients for all variables vs BMC at all skeletal sites.

BMC	FN	T	WT	L2-L4	L2	L3	L4
FN		0.2726 P=0.220	0.7855 **P=0.000**	0.7653 **P=0.000**	0.7294 **P=0.000**	0.6559 **P=0.001**	0.7799 **P=0.000**
T			0.3225 P=0.143	0.5512 **P=0.008**	0.5742 **P=0.005**	0.5583 **P=0.007**	0.4356 **P=0.043**
WT				0.5387 **P=0.010**	0.5556 **P=0.007**	0.4002 P=0.065	0.5501 **P=0.008**
L2-L4					0.9495 **P=0.000**	0.9451 **P=0.000**	0.9411 **P=0.000**
L2						0.8567 **P=0.000**	0.8374 **P=0.000**
L3							0.8307 **P=0.000**
L4							
SOC	0.0778 P=0.738	-0.3389 P=0.133	0.2576 P=0.260	-0.4080 P=0.066	-0.3353 P=0.137	-0.5206 **P=0.016**	-0.3221 P=0.154
UHPR	-0.2087 P=0.351	0.2958 P=0.181	0.0704 P=0.756	-0.0701 P=0.757	-0.0932 P=0.680	-0.0733 P=0.746	-0.0688 P=0.761
Area	0.1284 P=0.569	-0.2710 P=0.223	0.0580 P=0.798	-0.0322 P=0.887	-0.0848 P=0.707	-0.0807 P=0.721	0.0432 P=0.849
Time	0.0161 P=0.943	-0.2893 P=0.192	-0.0067 P=0.977	-0.0787 P=0.728	-0.0010 P=0.996	-0.1426 P=0.527	-0.0959 P=0.671
%Area	0.3925 P=0.071	-0.1465 P=0.515	0.3171 P=0.150	0.2410 P=0.280	0.1335 P=0.554	0.1718 P=0.445	0.3589 P=0.101
Weight	0.5953 **P=0.003**	0.0892 P=0.693	0.5263 **P=0.012**	0.4307 **P=0.045**	0.4060 P=0.061	0.4177 P=0.053	0.3845 P=0.077
Height	0.7302 **P=0.000**	0.4102 P=0.058	0.6546 **P=0.001**	0.6274 **P=0.002**	0.5650 **P=0.006**	0.6290 **P=0.002**	0.5711 **P=0.005**

APPENDIX XXVI (cont)

Correlation coefficients for all variables vs BMD at all skeletal sites.

BMD	FN	T	WT	L2-L4	L2	L3	L4
FN		0.7842 **P=0.000**	0.8853 **P=0.000**	0.5268 **P=0.012**	0.5864 **P=0.004**	0.4793 **P=0.024**	0.5463 **P=0.009**
T			0.6925 **P=0.000**	0.6848 **P=0.000**	0.7236 **P=0.000**	0.6403 **P=0.001**	0.6149 **P=0.002**
WT				0.4029 P=0.063	0.4468 **P=0.037**	0.3821 P= 0.079	0.3545 P=0.106
L2-L4					0.9051 **P=0.000**	0.9833 **P=0.000**	0.9332 **P=0.000**
L2						0.8492 **P=0.000**	0.8679 **P=0.000**
L3							0.8767 **P=0.000**
L4							
SOC	0.1683 P=0.466	-0.0351 P=0.880	0.2566 P=0.262	-0.2409 P=0.293	-0.1754 P=0.447	-0.2977 P=0.190	-0.2202 P=0.338
UHPR	-0.0084 P=0.971	-0.0851 P=0.707	0.0698 P=0.758	-0.2680 P=0.228	-0.1891 P=0.399	-0.2175 P=0.331	-0.3041 P=0.169
Area	0.1748 P=0.437	-0.1353 P=0.548	0.0594 P=0.793	-0.0813 P=0.719	-0.0840 P=0.710	-0.0909 P=0.687	0.0315 P=0.889
Time	0.0444 P=0.845	-0.0889 P=0.694	-0.0051 P=0.982	-0.0904 P=0.689	-0.0186 P=0.934	-0.1150 P=0.610	-0.0976 P=0.666
%Area	0.3517 P=0.108	0.0231 P=0.919	0.3217 P=0.144	0.1347 P=0.550	0.1303 P=0.563	0.0666 P=0.768	0.2625 P=0.238
Weight	0.3225 P=0.143	0.0613 P=0.786	0.5229 **P=0.013**	0.2229 P=0.319	0.1876 P=0.403	0.2642 P=0.235	0.1027 P=0.649
Height	0.5895 **0.004**	0.3214 P=0.145	0.6541 **P=0.001**	0.3695 P=0.091	0.3112 P=0.159	0.4301 **P=0.046**	0.2917 P=0.188

APPENDIX XXVII

Stepwise multiple regression of all post-test BMD and BMC sites using the independent variables of fitness, weight and height.

Dependent variable: BMD Femoral neck

Variable: Height

Multiple R	0.58952
R Square	0.34754
Adjusted R Square	0.31491
Standard Error	0.12036

Analysis of Variance

	df	Sum of Squares	Mean Square
Regression	1	0.15434	0.15434
Residual	20	0.28975	0.01449

F = 10.65313 Signif F = 0.0039

Variables in the Equation

Variable	B	SE B	Beta	T	Sig T
Height	0.015730	0.004819	0.589524	3.264	0.0039
(constant)	-1.744254	0.862100		-2.023	0.0566

Dependent variable: BMD Ward's triangle

Variable: Height

Multiple R	0.65407
R Square	0.42781
Adjusted R Square	0.39920
Standard Error	0.12822

Analysis of Variance

	df	Sum of Squares	Mean Square
Regression	1	0.24584	0.24584
Residual	20	0.32881	0.01644

F = 14.95331 Signif F = 0.0010

Variables in the Equation

Variable	B	SE B	Beta	T	Sig T
Height	0.019853	0.005134	0.654070	3.867	0.0010
(constant)	-2.487213	0.918369		-2.708	0.0135

APPENDIX XXVII (cont)

Dependent variable: BMD L3

Variable: Height

Multiple R	0.43005
R Square	0.18494
Adjusted R Square	0.14419
Standard Error	0.14262

Analysis of Variance

	df	Sum of Squares	Mean Square
Regression	1	0.09231	0.09231
Residual	20	0.40682	0.02034

F = 4.53819 Signif F = 0.0458

Variables in the Equation

Variable	B	SE B	Beta	T	Sig T
Height	0.012165	0.005711	0.430051	2.130	0.0458
(constant)	-1.075842	1.021509		-1.053	0.3048

Dependent variable: BMC Femoral neck

Variable: Height

Multiple R	0.73019
R Square	0.53318
Adjusted R Square	0.50984
Standard Error	0.40952

Analysis of Variance

	df	Sum of Squares	Mean Square
Regression	1	3.83100	3.83100
Residual	20	3.35419	0.16771

F = 22.84309 Signif F = 0.0001

Variables in the Equation

Variable	B	SE B	Beta	T	Sig T
Height	0.078371	0.016397	0.730192	4.779	0.0001
(constant)	-9.960426	2.933167		-3.396	0.0029

Dependent variable: BMC Ward's triangle

Variable: Height

Multiple R	0.65460
R Square	0.42850
Adjusted R Square	0.39993
Standard Error	0.12788

Analysis of Variance

	df	Sum of Squares	Mean Square
Regression	1	0.24524	0.24524
Residual	20	0.32708	0.01635

F = 14.99563 Signif F = 0.0009

Variables in the Equation

Variable	B	SE B	Beta	T	Sig T
Height	0.019829	0.005121	0.654599	3.872	0.0009
(constant)	-2.483567	0.915954		-2.711	0.0134

APPENDIX XXVII (cont)

Dependent variable: BMC L2-L4

Variable: Height

Multiple R	0.62735
R Square	0.39357
Adjusted R Square	0.36325
Standard Error	5.94532

Analysis of Variance

	df	Sum of Squares	Mean Square
Regression	1	458.79763	458.79763
Residual	20	706.93726	35.34686

F = 12.97987 Signif F = 0.0018

Variables in the Equation

Variable	B	SE B	Beta	T	Sig T
Height	0.857647	0.238053	0.627351	3.603	0.0018
(constant)	-104.53372	42.582745		-2.455	0.0234

Dependent variable: BMC L2

Variable: Height

Multiple R	0.56504
R Square	0.31927
Adjusted R Square	0.28523
Standard Error	2.23633

Analysis of Variance

	df	Sum of Squares	Mean Square
Regression	1	57.49168	57.49168
Residual	20	100.02367	5.00118

F = 9.38027 Signif F = 0.0061

Variables in the Equation

Variable	B	SE B	Beta	T	Sig T
Height	0.274247	0.89544	0.565041	3.063	0.0061
(constant)	-33.671323	16.017495		-2.102	0.0484

Dependent variable: BMC L3

Variable: Height

Multiple R	0.62902
R Square	0.39567
Adjusted R Square	0.36545
Standard Error	-2.09537

Analysis of Variance

	df	Sum of Squares	Mean Square
Regression	1	57.49168	57.49168
Residual	20	87.81165	4.39058

F = 13.09432 Signif F = 0.0017

Variables in the Equation

Variable	B	SE B	Beta	T	Sig T
Height	0.303599	0.083899	0.629020	3.619	0.0017
(constant)	-37.765365	15.007877		-2.516	0.0205

Dependent variable: BMD Femoral neck

Variable: Height

Multiple R	0.57114
R Square	0.32620
Adjusted R Square	0.29251
Standard Error	2.17843

Analysis of Variance

	df	Sum of Squares	Mean Square
Regression	1	45.94882	45.94882
Residual	20	94.91148	4.74557

F = 9.68246 Signif F = 0.0055

Variables in the Equation

Variable	B	SE B	Beta	T	Sig T
Height	0.271416	0.087225	0.571140	3.112	0.0055
(constant)	-31.522813	15.602802		-2.020	0.0569