LONGITUDINAL STUDY OF THE FACTORS WHICH AFFECT THE DEVELOPMENT OF BONE MINERAL CONTENT, BONE WIDTH AND BONE MINERAL DENSITY THROUGH ADOLESCENCE

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Thesis submitted for the degree of
Doctor of Philosophy
in
The University of Adelaide
(Faculty of Medicine)

TABLE OF CONTENTS

SUMMARY

This thesis presents prospective data on forearm bone status in a group of Australian children. Bone mineral content (BMC), width (BW), areal density (aBMD) and volumetric density (vBMD) were determined at ages 11, 13, 15 and 17 years in 56 boys and 52 girls. Absolute values of all bone variables increased with age and sexual maturity. Velocities were dependent on age and sexual maturity. Maximal increases in all bone variables occurred earlier in girls than in boys due to girls' earlier sexual maturity, and earlier in BW than in the other bone variables. vBMD velocity was negative in boys from 11 to 13 years. At 17 years BMC, BW and aBMD were significantly greater in boys than girls but there was no difference in vBMD.

Between 11 and 17 years BMC and vBMD as percentages of the young same sex adult means increased from 45 and 71% respectively to 86% for both in boys, and from 55 and 71% to 93 and 94% respectively in girls. Values at 17 years and bone velocities from 15 to 17 years suggested that girls were near peak bone status by age 17 but significant gains were still occurring in boys. Comparison of bone status according to bone age indicated that at cessation of longitudinal growth girls were very near peak bone status (97%) but increases were likely to continue in boys.

Neither nutrient intake nor physical activity was detectably correlated with bone status or bone status velocity. However BMC, aBMD and vBMD velocities from 11 to 17 years were significantly greater in those girls with consistently high calcium intakes (>RDI) than those with consistently low intakes (<0.7RDI). The high degree of tracking in all bone variables suggested that there was limited opportunity for environmental factors to alter bone status.

Multiple regression analysis determined the ability of biological factors, environmental factors and genetic factors, to predict each bone variable and the change in each variable. 80%, 71% and 49% of the variance of BMC, BW, and BMD respectively was accounted for by a combination of up to eight variables. 52% of the variance in change in BMC, 55% for BW,

and 58% for BMD was accounted for by a combination of up to five factors. Neither calcium intake nor physical activity were significant variables in any equation.

The stronger correlation of bone variables between both sons and daughters and their mothers, compared with their fathers and the lower bone status velocity in girls with consistently low calcium intakes, identifies a target population (girls with poor calcium intake and daughters of osteoporotic mothers and grandmothers) for further investigation. Meanwhile the public health messages of the benefits of good nutrition and regular physical activity should be targetted at children before and during their period of rapid growth so that their genetic potential of peak bone status can be achieved.

This work contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

SIGNED: DATE: 10/4/97

ACKNOWLEDGMENTS

The subjects of the present study are a subset of a larger longitudinal study initiated at birth of the subjects, in 1975-1976. I have been involved in that study since the subjects were four years old. Growth and nutrient intake have been determined throughout childhood and the families assessed for coronary heart disease risk factor precursors when the subjects were aged 8 years. Publications resulting from this study are listed in the appendix.

Most importantly I must acknowledge the commitment of the boys and girls and their parents, who are the subjects of this study. The children were willing participants but without the support of their parents, who ensured the diet records and questionnaires were completed and the children attended for measurements, much data would be missing. I enjoyed the biennial contact with these families, following the children's progress through high school and watching them become young adults.

Dr John Boulton initiated the original study of these children in 1975. He has been a friend and mentor over many years. As principal supervisor he was always enthusiastic, ready with advice and tremendously encouraging. Despite the distance between us he made regular contact by phone, fax or in person.

Dr Bruce Foster (Department of Orthopaedics, Women's and Children's Hospital) smoothed my path in the initial phases. He was always available and supported and encouraged me whenever required. Provision of desk space in the department was invaluable. Thanks are also due to Dr Jim Penfold (Department of Paediatrics, University of Adelaide) my third supervisor. Staff in both these departments provided a friendly working environment.

Professor Chris Nordin suggested what has been an interesting study and was extremely supportive throughout. Dr Barry Chatterton of the Department of Nuclear Medicine, Royal Adelaide Hospital made the densitometer available for the bone measurements, Mr Chris

Schultz provided invaluable technical assistance and Ms Pam Francis assisted with the bone measurements. The staff of that department always made me and the children welcome.

Dr Lloyd Morris and staff of the Department of Organ Imaging, Women's and Children's Hospital performed and examined the hand radiographs for estimation of bone age.

Ms Jillian Rogers performed the diet analysis using the Diaryan program, prepared data from the larger Adelaide Nutrition study data base and gave miscellaneous computing advice as required. Dr David Bates (Department of Paediatrics, University of Adelaide) always cheerfully and immediately solved computing problems however trivial and Dr Alan Staples (Department of Genetics, Women's and Children's Hospital) advised on the multiple regression analysis.

Ms Jane Freemantle collected the blood specimens and Mr Alan Gilmore and staff of the Department of Endocrinology, Queen Elizabeth Hospital performed the hormone analyses.

Financial support was provided by a grant from NHMRC for the data collection at 11, 13 and 15 years and from Kelloggs Australia for analysis of the 17 year dietary intake data.

Finally I must thank my family who provided unwavering emotional support and ensured I knew there were other activities to be enjoyed.

ABBREVIATIONS

aBMD	areal bone mineral density
AN	anorexia nervosa
BMC	bone mineral content
BMD	bone mineral density
BMU	basic multicellular unit
BW	bone width
DPA	dual photon absorptiometry
DXA	dual x-ray absorptiometry
DZ	dizygotic
FN	femoral neck
FS	femoral shaft
LS	lumbar spine
MZ	monozygotic
PBM	peak bone mass
QCT	quantitated computed topography
QMD	quantitated roentgen microdensitometry
RDA	recommended dietary allowance
RDI	recommended dietary intake
SPA	single photon absorptiometry
TB	total body
TB BMC	total body bone mineral content
TB BMD	total body bone mineral density
vBMD	volumetric bone mineral density
VDR	vitamin D receptor

CHAPTER 1

INTRODUCTION

Childhood and adolescence are times of considerable bone growth. By the end of the second decade at least 90 percent of adult bone mass has been attained. The maximum bone mass an individual attains is their peak bone mass (PBM). The importance of maximising PBM has received increased attention in recent years. The risk of osteoporotic fracture at any site is inversely related to the bone density at that site and the amount of stress applied to that bone (Eisman et al 1993). The bone density at a given age at any site in the skeleton is the consequence of PBM and the subsequent rate of bone loss from that site in later life. Thus PBM is a major determinant of the risk of osteoporosis.

Osteoporosis is the clinical expression of bone loss. Because of our ageing society and increasing life expectancy it is an increasing health care problem (Cooper et al 1992, Melton 1993). As a major cause of fractures in post-menopausal women and elderly men, it results in considerable morbidity and mortality in all developed countries including Australia (Salkeld and Leeder 1990). Current treatment for osteoporosis is unable to reverse bone loss to any substantial degree, at best it can halt further loss (Consensus Development Conference 1993). Therefore probably the most effective measure in the treatment of osteoporosis is prevention by ensuring achievement and maintenance of maximal PBM before the onset of the natural process of bone loss.

As some 40% of adult bone is accumulated in the adolescent years this is a critical time for optimising bone mass (Landin and Nilsson 1981, Geusens et al 1991). In the mid 1980's when facilities for measurement of forearm bone density were available in Adelaide, only four studies of bone mass estimation in children had been published (Christiansen et al 1975a, 1975b, Krabbe et al 1979, Landin and Nilsson 1981, Hui et al 1985), and only one longitudinal study on a group of 11 to 13-year-old boys (Krabbe and Christiansen 1984).

In 1986-87 a group of normal healthy Adelaide children, who had been participating in a study of growth and nutrition since birth, turned 11 years of age. These children were invited to participate in a study of forearm bone mass and density throughout adolescence.

In the 1990s, developments in bone density technology, led to publication of a wealth of studies, but Australian studies remain scarce (Lu et al 1994, 1995, Henderson et al 1995). Apart from a subset of 55 subjects followed up at one year in the study by Lu (1994), there have been no longitudinal studies in Australian children. The results of the Adelaide longitudinal study are the basis of this thesis.

The literature review (chapter 2) provides a background to studies of bone growth in children and adolescents. In section 2.1 the composition of bone, the growth of bone through childhood and the evidence for the timing of PBM are described. The relevance of PBM to osteoporosis is discussed in section 2.2. In the following section (2.3) the evidence for the role of genetic and environmental factors in determining PBM is reviewed. In section 2.4 the measurement of bone status is described. The final two sections review studies of bone status in children and adolescents (2.5) and describe the effects of the biological factors of age, gender, growth and maturity on bone status in children and adolescents (2.6).

Chapter 3 describes the methods of the study and in the following four chapters (4-7) the results are presented. In chapter 4, bone size, mass and density and rate of change in these variables are related to the biological factors age, gender, pubertal status and anthropometric measures. The presentation of the results follows the conventional approach in child health research of using chronological age as the starting point. Data are subsequently presented according to other measures such as pubertal status and skeletal maturity. Age is a readily determined variable, data on pubertal status may often be unavailable.

In chapter 5, nutrient intake and physical activity level at each age and the relationship of these environmental factors to bone size, mass and density and rate of change in these variables are described. In chapter 6 parental values of bone size, mass and density are presented.

Children's values are related to these parental values and heritability estimated. In the final results chapter, 7, multiple regression analyses, to determine the significant predictive factors of absolute bone size, mass and density and velocity of these variables, are presented.

CHAPTER 2

LITERATURE REVIEW

2.1 BONE GROWTH

2.1.1 Composition of bone

Bones comprise two components - compact (cortical) and cancellous (trabecular) bone. The cortical bone provides a dense shell around all bones and thereby gives support to the internal trabecular network and at the same time encloses the bone marrow. Cortical bone constitutes approximately 80% of the skeleton. In the diaphyses of long bones the cortical bone is very thick and gives these bones the extreme strength to act as a load bearing frame for the muscular system. At the metaphyses and other places e.g. the vertebral bodies, the femoral neck, and the ultra-distal part of the radius and ulna, the cortical bone is only a thin shell. At these sites trabecular bone, which constitutes only 20% of total bone mass is dominant (Mosekilde 1993).

Bone tissue consists of 65% mineral and 35% organic material. The organic matrix comprises 90-95% collagen fibres which extend primarily along the lines of tensional force and give bone its powerful tensile strength. The remaining 5-10% is ground substance which is extracellular fluid plus proteoglycans. The inorganic components of bone are mainly calcium hydroxyapatite crystals and amorphous calcium carbonate plus small amounts of magnesium hydroxide, fluoride, sulphate, sodium and potassium. As these minerals are deposited in the framework formed by the collagenous fibres the intercellular substance of the tissue hardens, that is becomes calcified.

2.1.2 Bone growth

The human skeleton is formed and modelled during infancy, childhood, and youth under the influence of mechanical loading. The skeleton grows in length from the specialised cartilage of the epiphyseal plates and the bones expand in diameter by subperiosteal bone formation from the cambium cells. Bones achieve their characteristic external shape through the process

of modelling. During bone modelling osteoblasts and osteoclasts work independently and on different bone surfaces. The net balance is positive and in young adulthood, bones reach their final external form and the skeleton achieves its maximum mass and strength (Mosekilde 1993). At the same time as bones are growing in size bone remodelling occurs via the basic multicellular unit (BMU) (Jaworski 1981). This remodelling ensures that bone shape and architecture are preserved despite the increase in size. It contributes to the gross organisation of bone and accounts for the microscopic organisation as well as the adaptation of bones to their function of providing physical support.

Calcium forms an integral part of the skeleton. It is the fifth most abundant element in the human body and makes up 1.9% of the body by weight. Over 99% of body calcium is in the skeleton. At birth the human skeleton contains about 25 g calcium and at maturity 1000 to 1200 g. Calcium contributes not only to structural support but acts as a large reservoir for maintaining the plasma calcium concentration at a very stable level.

During the first year of life linear velocity is greater, and the calcium content of the body increases faster in relation to body size, than at any other time in the life cycle. The skeletal mass doubles in this first year requiring a calcium retention of 100 mg per day. As linear velocity declines retention of calcium also declines slightly to a level between 80 and 100 mg per day through childhood (American Academy of Paediatrics 1978). The periods preceding puberty and during puberty (9 - 17 years), are characterised by accelerated muscular and skeletal growth.

Approximately 40% of the total skeletal mass of adults is accumulated during this period of rapid growth in the second decade of life. At this time the bone mineral content increases at a rate of approximately 8.5% per year and calcium needs are greater than in either childhood or adulthood. Calcium retention reaches a peak of 290 to 400 mg per day in boys and 210 to 240 mg per day in girls, or an average daily increment of 180 to 210 mg in boys and 90 to 110 mg in girls throughout adolescence (American Academy of Paediatrics 1978).

2.1.3 Peak bone mass (PBM)

The consolidation of bone begins in adolescence and continues into young adulthood after linear bone growth has ceased. The final maximum bone mass acquired in adulthood is called the peak bone mass (PBM) and depends on the size of the bone and its density. Bone mass is determined from the mineral content of the bone (assuming full mineralisation) and is expressed as bone mineral content (BMC), which is bone mineral per linear measure (g/cm) in the forearm, or total bone mineral (g) in the area of the scan, eg in the spine or proximal femur. Areal density (aBMD) (g/cm^2) is obtained by dividing the first BMC by bone width or the second BMC by the area under the scan. These are not true volumetric densities (g/cm^3, mg/ml) because the third dimension is missing. True volumetric bone density is obtained by dividing the first BMC by the cross-sectional area of the bone as in the forearm or femoral shaft.

BMC will increase as bone size increases; a young adult male with a larger skeleton than a young adult female will have a greater BMC. Within the same gender a person with large bones will have a greater BMC than another person with smaller bones. BMC will therefore increase as stature increases and as the bones expand in diameter, but will also increase as the mineral density of the bone increases, ie when there is an increase in bone tissue per volume of bone organ. Bone mineral density expressed as an areal measure takes account of the width of the bone or the projected area of the scan, but not the third dimension (depth), and thus will vary with bone size. In the majority of studies aBMD is used as the measure of bone density, but may overestimate vBMD in large bones and underestimate vBMD in small bones (Carter et al 1992). The volumetric measure of bone density (vBMD) takes account of the volume of the bone measured and therefore is totally independent of bone size. vBMD will not change if bone diameter is increasing and the new bone is of the same density, but there will be a small increase in areal bone density. vBMD will increase if bone diameter is not changing but the bone tissue per volume of bone organ is increasing, and similarly volumetric bone density will decrease if there is loss of bone although the external diameter of the bone is unchanged. Thus detection of changes in bone density as distinct from changes in bone size, requires estimate of vBMD.

Bone gain or loss is used to refer to an increase or decrease in BMC or BMD. Similarly whilst PBM strictly refers to peak bone mineral content the term is used loosely to describe maximal attainment of BMC or BMD (areal or volumetric). The three measures are not interchangeable.

The precise age at which PBM is attained remains uncertain. Trotter and Hixon (1974) studied the weight, density and percentage ash weight of the human skeleton from 16 weeks gestation to 100 years of age and this work indicated that it was near the end of the third decade. These observations were supported by the radiogrammetry studies of Garn (1970) who demonstrated bone mass gains through the third decade from measurements of metacarpal lengths, and cortical diameters and areas taken from radiographs. More recently Gilsanz et al (1988a) compared the trabecular vertebral density of two groups of women: adolescents aged 14 to 19 years and young adults aged 25 to 35 years. The adolescent group had a significantly higher mean density suggesting that spinal density reaches its peak around the time of cessation of longitudinal growth near the end of the second decade. (This study may be affected by cohort effects and differences in body size)

Many cross-sectional and several longitudinal studies of bone mass and density by various densitometric methods have attempted to answer this question of the timing of peak bone mass. These studies have been predominantly on females. The majority report bone mineral content and areal bone density, a few report volumetric bone density. Cross-sectional studies of females are summarised in Table 2.1 and those of males in Table 2.2, and longitudinal studies are summarised in Table 2.3. All these post-date the time of commencement of the present study.

Cross-sectional studies covering the age range 15-50 years have suggested that PBM is attained near the end of the second decade These studies have used a variety of techniques. Studies using single photon absorptiometry (SPA) to measure forearm bone density have included that of Sowers et al (1985) who found no significant change in forearm bone mass values across the 15-year age span of 20 to 35-year-old women. Similarly Mazess and Barden

(1991) in a cross-sectional study of 225 women age 20 to 39 years found no age-related change in forearm BMD and Matkovic et al (1994) in a study of 265 girls and premenopausal women aged 8 to 50 years reported that most of the bone mass of the forearm was accumulated by 18 years after which it increased very slowly. Halioua and Anderson (1990) reported from measurements in 181 Caucasian women aged 20 to 50 years that BMC, BW and BMD of the mid-radius remained the same over the three decades suggesting that PBM of this site was achieved in late adolescence. However BMC, BW and BMD of the distal radius increased significantly up to the end of the fourth decade suggesting PBM for this site was achieved sometime in the 30's. These studies on the forearm indicate that the majority of bone mass at this site is attained by the end of the second decade but that small increases may occur in subsequent years. A decline in forearm bone mass was not observed before 50 years of age.

Studies using dual photon absorptiometry (DPA) have measured the axial skeleton. Mazess and Barden (1991) found no age-related change in axial (spine) bone mineral density in 225 women age 20 to 39 years, a similar finding to that for the forearm. Gordon et al (1991) in their study of 117 females aged 3 to 30 years found that BMC and BMD of the lumbar spine (LS) did not change after 15 years. Using dual X-ray absorptiometry (DXA) to measure bone mass at multiple sites and the total body, Rico et al (1993b) found no change with age across five-year age groups in total body bone mineral content (TB BMC) and density (TB BMD) and regional (axial and peripheral) bone mineral content and density in premenopausal women aged from 15 years. Lu et al 1994 studied 130 females aged 4 to 27 years and reported TB BMC and TB BMD and LS BMD peaked at 15.8 years whereas femoral neck (FN) BMD peaked at 14.1 years. Whilst TB BMD did not increase beyond the inflection point, BMD of LS and FN decreased. Teegarden et al (1995) in a study of 247 females aged 11 to 32 years estimated that 90% of TB BMD was attained by age 13.6 years and 99% by age 22.1 years whereas equivalent percentages for TB BMC were attained several years later at 16.9 and 26.2 years respectively. Matkovic et al (1994) in a study of 265 girls and premenopausal women aged 8 to 50 years reported little change in total body BMC and BMD after 18 years a similar finding to that for the forearm but that PBM (BMC and BMD) of the

Table 2.1

Cross-sectional studies in females to determine timing of PBM

Author, year	Subjects		Site measured[a]	Bone measure[b]	Result
	N	age			
Sowers et al,1985	86	20-35	forearm	BMD	no change across age range
Halioua & Anderson, 1990	181	20-50	distal radius, mid-radius	BMC, BW, BMD	distal radius increased up to 40 years
Mazess & Barden, 1991	225	20-39[c]	LS, femur, humerus, radius	BMC, BMD	no change across age range
Gordon et al, 1991	117	3-30	LS	BMC, BMD	no change after 15 y
Rico et al, 1993b	242	15 +[c]	TB, head, trunk, arms,legs	BMC, BMD	no change across age range
Matkovic et al, 1994	265	8-50	LS, proximal femur	BMC, BMD[d]	declined after 15y
			TB, radial shaft, distal forearm		no change
Lu et al, 1994	130	4-27	TB, LS	BMC, BMD	increased to 15.8 y
			FN	BMC, BMD	increased to 14.1 y
Lu et al, 1995	100	8-27	FS	vBMD	no change across age range
Teegarden et al, 1995	247	11-32	TB	BMC, BMD	99% BMC at 26.2 y, BMD 22.1 y

a: LS lumbar spine; TB total body; FN femoral neck; FS femoral shaft

b: BMD as areal measure unless otherwise indicated

c: 5-year age groups

d: estimated bone volume to give volumetric density for LS only

Table 2.2
Cross-sectional studies in males to determine timing of PBM

Author, year	Subjects		Site measured[a]	Bone measure[b]	Result
	N	age			
Rico et al, 1992	121	15-29[c]	TB, head, trunk, arms, legs	BMC, BMD BMC	increased to 24 y
Gordon et al, 1991	129	3-30	LS	BMC, BMD	increased across age range
Lu et al, 1994	136	4-27	TB, LS, FN	BMC, BMD	increased to 17.5 y
Lu et al, 1995	100	8-27	FS	vBMD	no change across age range

a: LS lumbar spine; TB total body; FN femoral neck; FS femoral shaft
b: BMD as areal measure unless otherwise indicated
c: 5-year age groups

Table 2.3
Longitudinal studies in males and females to determine timing of PBM

Author, year	Length of study	Subjects N	Subjects age	Subjects sex	Site measured[a]	Bone measure[b]	Result
Davies et al, 1989	up to 4 years	191	18.5-25	F	LS, forearm wrist	BMC, BMD BMC, BMD	increased across age range no significant change
Mazess & Barden, 1991	2 years[c]	235	20-39(a)	F	LS, femur, humerus, radius	BMC, BMD	no significant change
Recker et al, 1992	up to 5 years	156	18.5-26	F	LS, forearm TB	BMC, BMD BMC	increased across age range increased across age range
Theintz et al, 1992	1 year	100, 98	9-19	M, F	LS, FN, FS	BMC, BMD	females: no significant change after 17 y males: increased across age range

a: LS lumbar spine; TB total body; FN femoral neck; FS femoral shaft
b: BMD as areal measure unless otherwise indicated
c: 5-year age groups

trabecular bone of the spine and proximal femur was attained at 18 years and started to decline immediately after that age. In this study BMD measurements of the lumbar spine were estimated as volumetric density compared with the other studies in years and started to decline immediately after that age. In this study BMD measurements of the lumbar spine were estimated as volumetric density compared with the other studies in which areal density only was estimated. One other study has estimated true volumetric BMD. Lu et al (1995) assumed the femoral shaft (FS) to be a cylinder and thus determined vBMD in 100 females aged 8 to 27 years. They found no change in vBMD across the entire age range. This finding indicates the importance of clearly defining what measurement is being made namely BMC, aBMD or vBMD when describing PBM.

These cross-sectional studies show consistent results irrespective of the method of measurement (SPA, DPA, DXA) used to determine bone mass. They demonstrate that although most of the bone mass at all sites is attained near the end of the second decade, there are differences in timing of PBM depending on the type of bone. Trabecular bone mass (LS, FS) peaks in the late teens and then declines. Most of the cortical bone mass is accumulated by the late teens, but in some cases it continues to increase after that time but at a very slow rate. That some studies have shown no increase in cortical bone mass and others have shown small increases in the third and fourth decades may be due to cohort effects and differences in sample size with small increases being difficult to detect when there is such a wide range of values (type II error).

Fewer studies have included males, but these have generally shown that PBM is attained at a later age than in females (Table 2.2). Rico et al (1992) studied males aged 15 to 29 years and found a significant positive correlation between age and total body bone mass determined by DXA. From these findings they concluded that women reach peak bone mass earlier than men, and that in women this occurred in late adolescence. Gordon et al (1991) in a study measuring LS BMD by DPA in 129 males aged 3 to 30, reported that BMD was still increasing up to the age of 30 years, whilst Lu et al (1994) from a study of 136 males aged 4 to 27 years reported that BMD of TB, FN and LS, measured by DXA, peaked at 17.5 years, 1.7 years later than in

females. Whilst TB BMD continued to increase slowly after this inflection point, LS and FN BMD declined, a similar pattern to that observed in females.

Longitudinal studies can determine more accurately whether bone mass is increasing or decreasing by eliminating inter subject variation. Such studies may also eliminate the problem of truly defining PBM when bone mass is dependent on body size and size tends to increase with age (Sowers et al 1991).

One longitudinal study suggests that PBM is attained in the third decade. This study on women aged 18 to 25 years, spanning up to 4 years, showed that the women were still gaining bone in the third decade in both the axial (determined by SPA) and appendicular (determined by DPA) skeleton (Davies et al 1989). Recker et al (1992) reported results from the same study after 5 years. They estimated an increase in bone mass in the third decade of between 4.8 and 12.5% depending on the site. Additionally they showed that rate of gain in bone mass decreased with age and predicted an endpoint for bone gain close to 30 years of age. In contrast Mazess and Barden (1991) measured a subset of their female sample up to 2 years after the initial measurement and reported no change in BMC or BMD across the age range 20 to 39. Theintz et al (1992) studied females aged 9 to 19 years. Although unable to determine the exact time of PBM they reported that in females BMC and BMD of LS and FN, determined by DXA, increased from 11 to 14 years, but after 16 years the rate of increase fell to the point at which gains were not statistically significant over a one-year interval. The shorter time of these two latter studies are the possible reason for not detecting significant changes in bone mass in subjects older than 16 years.

Only one longitudinal study has included males. Theintz et al (1992) studied males aged 9 to 19 years over a one year period. They found that the incremental gains in BMC and BMD of LS and FN were most marked between 13 and 17 years after which the rate declined, but remained significant between 17 and 20 years.

These longitudinal studies confirm the findings from cross-sectional studies of a rapid gain in bone mass in the early adolescent years with the most rapid gains occurring in females at an earlier age than in males. The longitudinal studies of shorter duration indicate that following this rapid increase there is no further significant increase in females. However the study of Recker spanning 5 years showed that bone mass continues to increase, a finding consistent with some cross-sectional studies but at variance with others. The age range of the males was not sufficiently great to determine if bone mass increases ceased after 20 years.

Of particular interest is how bone mass continues to increase in the third decade in a period following cessation of longitudinal growth which was determined radiologically by closure of the epiphyseal plates. Katzman et al (1991) suggested that endosteal bone apposition may occur as red marrow is lost during maturation whilst Matkovic et al (1994) suggested that this gain is probably the consequence of periosteal expansion. Bone gain may also result from consolidation of trabecular bone.

To determine precisely the age of PBM longitudinal studies are required in which stabilisation of bone mass is demonstrated. As total bone mineral and bone mineral density are relevant to risk of fracture following loss of bone in later life, both BMC and volumetric BMD need to be assessed.

Not only do males appear to reach PBM at a later age than females there are also differences in actual bone mass between males and females with males having a greater TB BM than females due to their greater stature. Mazess (1982) estimated from published studies that premenopausal women had 30% less cortical bone mass than men, but that this difference halved to only 15% when adjusted for bone size (there was no indication if this measure of bone size was areal or volumetric). A study of 29 dizygotic twins of differing within-pair sex showed that the men had higher areal bone densities of the radius compared with their female twins, but the females had a greater areal bone density at the LS and FN (Kelly et al 1990c). These regional differences in bone density may be due to the effects of minor biomechanical

differences in the lower back and pelvis, together with the effects of muscle strength and arm use in males.

The differences in aBMD between males and females which are due to the greater bone size in males appear before puberty. Gilsanz et al (1994) using quantitated computed topography (QCT) showed that vertebral body size in children and adolescents was greater in boys and concluded that the lower vertebral bone mass in women may result from gender differences in bone size. Lu et al (1994) reported that males had a higher peak TB BMD but there was no difference in LS BMD, a finding that conflicts with that of Kelly et al (1990c). This sex difference in TB BMD could be explained by the differences in lean tissue mass and weight between the males and females indicating the dependence of TB BMD on size.

Comparison of BMD between sexes requires measurement of vBMD which can be determined using QCT or from SPA and DXA measures using estimated cross-sectional area of bone as is possible in the longitudinal bones of the forearm and leg (Nordin and Polley 1987, Lu et al 1995).

In summary over 90% of bone mineral is attained by about age 17 years of age in girls and up to two years later in boys. There are site differences in this timing with predominantly trabecular sites such as LS reaching maximal mass earlier than predominantly cortical sites. Bone gain continues in the third decade at most sites but at a much slower rate than in the second decade. Boys have a greater peak bone mineral than girls due principally to their greater bone size. When bone mineral is expressed as a volumetric density much of the gender difference is lost.

2.2 PBM IN THE AETIOLOGY OF OSTEOPOROSIS

Osteoporosis is an increasing health care problem world wide (Cooper et al 1992, Melton 1993). It is a major cause of fractures in postmenopausal women and elderly men. The incidence of fractures in most societies is increasing (Zetterberg and Anderson 1980, Lewis

1981, Wallace 1983, Johnell et al 1984, Spector et al 1990, Rockwood et al 1990, Johnell et al 1992, Lau 1993, Gullberg et al 1993). This increase is due in part to the ageing of these populations as a result of increased life expectancy and demographic changes, but also may be explained by an increasing hip axis length (Reid et al 1994). Osteoporosis is characterised by decreased bone mass/density at multiple sites and architectural abnormalities in trabecular bone (Parfitt 1987), resulting in decreased bone strength. The disorder is difficult to detect early in its development because bone loss progresses gradually over many years without symptoms until fracture occurs. When bone loss is sufficiently great fractures may occur spontaneously or after minimal trauma, particularly in the spine. In the elderly, osteoporosis and related fractures are a major cause of morbidity and mortality. Although vertebral fractures cause pain and skeletal deformity, hip fractures are more serious. In Australia the incidence of atraumatic fractures in those over 60 years of age has been estimated as 1.9% in men and 3.1% in women (Nguyen et al 1993). In 1986, 416 000 bed days were occupied for osteoporotic fractures and the annual hospitalisation and nursing home costs of osteoporosis in 1988/89 were estimated as 172 million dollars (Salkeld and Leeder 1990).

After a hip fracture the lifestyle of patients is considerably altered with many of them requiring nursing home care, or at least dependence on others on a daily basis. The risk of osteoporotic fracture at any site is related to the bone mineral density at that site and the amount of stress applied to that bone (Eisman et al 1993). With ageing, falls may become of increasing importance as a mechanism whereby excessive stresses are applied suddenly to certain bones. Individuals with high bone density are much less likely to suffer a fracture, although not all people with low bone density will necessarily sustain a fracture from a given stress, or spontaneously in the case of vertebral fractures. Thus bone density is a determinant of fracture risk ie the lower the bone density the greater the fracture risk. Cummings et al (1990) reported that the risk of hip fracture was inversely related to bone density measured by SPA at three sites in the forearm. They estimated that a bone density one standard deviation below the mean increased the risk of fracture by 50%.

Cleghorn et al (1991) related fracture history to forearm BMD in 492 normal post-menopausal women. Data is shown in Figure 2.1 as fracture rate as a function of the density expressed in standard deviation units. The adjusted relative risk of fracture (fitted odds ratio) as a function of bone density was determined from logistic regression (Figure 2.2). Subjects with a forearm bone density more than four standard deviations below the mean for young adults had an increased risk of fracture of 5.5 (2.7 - 11.4, 95% confidence limits).

The bone density at any site is the consequence of the peak bone mineral density achieved at that site in young adulthood and the subsequent rate of bone loss from that site in later life. For both men and women, at each bone site, there is a wide normal range in peak bone density and for women this wide range is also seen at menopause. Thus at that stage the low-normal individual may already be at the upper limit of the fracture threshold. The rapid bone loss which occurs in women in the early years post-menopause will quickly bring such an individual below the fracture threshold. The slower but continuous age-related bone loss common to both sexes will take longer to manifest as a bone mass below the fracture threshold.

Although osteoporosis can affect all bones, the common fracture sites are those at which trabecular bone is dominant, for example the vertebral bodies, femoral neck and the ultra-distal radius and ulna.

Peak bone mass/density and rate of bone loss are the major determinants of bone mass and density in the elderly. Hui et al (1990) showed that until at least 15 years after the menopause, peak bone density was more closely associated with later bone density than was the rate of bone loss from the menopause. Nordin (1993) however showed that the proportion of the variance in forearm BMC at 5-year intervals after the menopause accounted for by the initial value fell from 91.8% at 5 years, to 67.9% at 10 years and 45.6% at 15 years. He concluded that by 15 years post-menopause, the rate of loss rather than the initial value was the major determinant of bone mass and that the predictive value of a single bone mass/density measurement did not exceed 5 years.

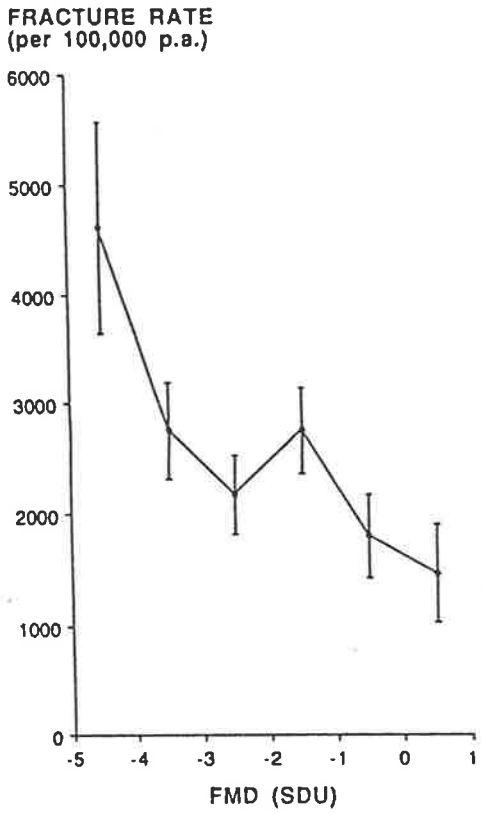


Figure 2.1 Fracture rates (±SD) as a function of forearm mineral density (FMD) expressed in standard deviation units.

(From Cleghorn et al 1991 with permission from Calcif Tissue Int)

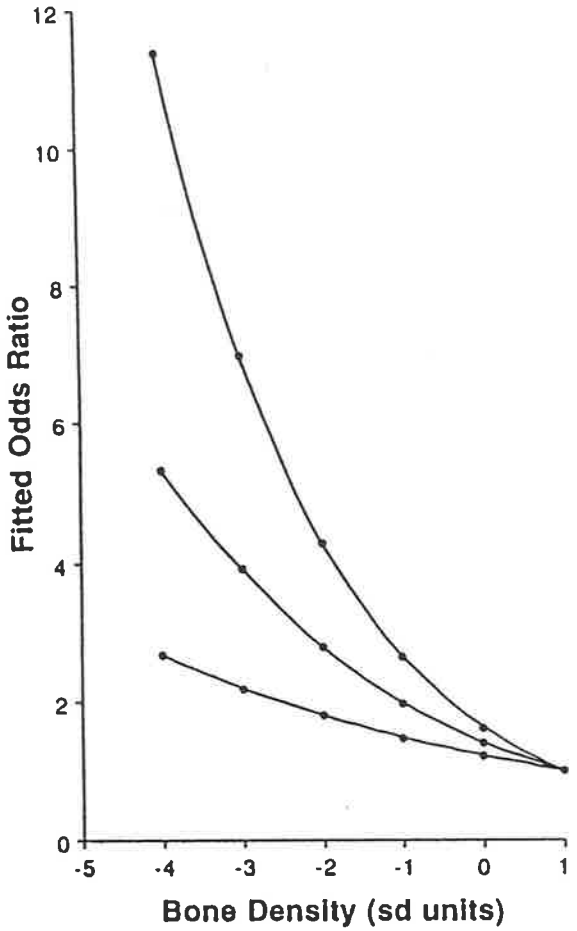


Figure 2.2 Fitted odds ratios (representing adjusted relative risk) as a function of forearm bone density. The top and bottom lines represent the 95% confidence limits.

Despite this increasing importance of bone loss on bone mass/density in the elderly after 5-years postmenopause, an optimal premenopausal bone density is probably the best preventive measure against clinically relevant postmenopausal and age-related osteoporosis (Eisman 1991). The importance of this is further enhanced by the present inadequacy of treatment regimens of established osteoporosis. Current therapies are able to prevent further bone loss, but it is difficult to restore lost bone (Consensus Development Conference 1993). Current therapies fall short of rapid, sustained benefit with negligible side effects or ease of use enhancing compliance (Eisman 1995a).

Therefore achievement by an any individual of his/her potential PBM and maintenance of that peak density are probably the most effective options for prevention of low bone mass and future osteoporosis. The optimal development of bone mass in early life involves a series of events partly mediated by ethnic and genetic factors, but also substantially modified by dietary and lifestyle factors (Eisman et al 1993).

2.3 PBM AND GENETIC AND ENVIRONMENTAL FACTORS

2.3.1 Genetic factors

Evidence for a genetic component to bone composition and mass comes from many areas of study: comparisons between ethnic groups (Garn 1970, Trotter and Hixon 1974, Pollitzer and Anderson 1989), sex differences (Trotter and Hixon 1974, Rico et al 1992, Seeman 1993, Kelly et al 1990c), mother/parent-daughter comparisons (Matkovic et al 1990, Lutz 1986, Tylavsky et al 1989, Seeman et al 1989, Krall and Dawson-Hughes 1993, McKay et al 1994), twin studies (Dequeker et al 1987, Pocock et al 1987, Christian et al 1990, Kelly et al 1990a, Kelly et al 1993, Slemenda et al 1991a, Takeshita et al 1992), and most recently identification of a vitamin D receptor gene (Morrison et al 1994). These studies demonstrate that inherited familial and ethnic factors operate throughout life to determine peak bone mineral density and its subsequent rates of change, through sensitivity of bone to environmental factors (Eisman et al 1993).

Garn (1970) showed differences in bone mass between Afro-Americans (Blacks) and Whites, with the former having more cortical bone area than the latter. Trotter and Hixon (1974) showed that Blacks' skeletons exceed Whites' skeletons, and in those over 40 years, males exceed females, in mean weight and density, and to a lesser degree in percentage ash weight.

Pollitzer and Anderson (1989) reviewed the literature on bone measurements in different ethnic groups and populations. They concluded that even after adjustment for body mass there were differences between Blacks and Whites in bone density. McCormick et al (1991) described ethnic and sex differences in spinal bone mineral density in Black, White, and Hispanic children and adolescents, and Gilsanz et al (1991) reported that differences in vertebral bone density between Black and White females occurred in a relatively brief period in late puberty. However, Patel et al (1992) found no significant differences in appendicular bone mass of Black and White children. A study by Russell-Aulet et al (1991) of White and Asian men showed the Asians had lower total body bone mineral mass and BMD in all the seven regions measured, but that when these measurements were adjusted for height and weight there were no significant differences in bone measurements. In these studies BMD was expressed as an areal density (aBMD) and thus did not take into account the depth of the bone through which the beam is passed. The larger the skeleton the greater is the depth and the greater the aBMD appears (Carter et al 1992). Artefactual differences in aBMD between ethnic groups could therefore arise if the skeletal dimensions of the groups differ. To correct for the effects of differing skeletal size Cundy et al (1995) assumed the vertebral body to be a cube of uniform mean dimensions and thus estimated a volumetric BMD (vBMD). They compared Chinese, Indian, European and Polynesian women and found no difference in vBMD between European, Indian and Chinese women, but that Polynesian women had significantly higher vBMD than European, Indian and Chinese women. They concluded that differences between Europeans and Asians were largely due to a measurement artefact arising from use of aBMD rather than vBMD, but that there was a true difference in vBMD between Polynesians and the other ethnic groups.

Familial resemblance in bone density has been demonstrated in a number of studies. Matkovic et al (1990) presented data on 14 year old females that suggested that bone mass attainment is under the genetic influence of both parents. The strongest correlations were between daughters' and parents' mean values. Similar findings were noted by Lutz (1986) and Tylavsky et al (1989) who showed significant relationships between mother-daughter pairs in bone mineral content and density. Seeman et al (1989) reported that daughters of women with fractures had significantly lower bone mass at the lumbar spine and femoral neck than daughters of mothers without fractures. Krall and Dawson-Hughes (1993) measured bone mineral density at five sites in 160 members of 40 families. After adjusting for age, height, and weight and significant life-style and environmental factors they estimated that 46-62% (depending on the site) of the variance in bone density was attributable to heredity. A recent study by McKay et al (1994) measured BMD at the lumbar spine and proximal femur in three generations of families. They found strong BMD Z-score correlations, ranging from 0.41 to 0.57, between mother-grandmother and mother-daughter pairs. There was no significant association between the boys and their mothers which the authors suggested may have been due to the lower maturity status of the boys compared with the girls. Studies determining associations between family members measure the overall familial resemblance within the family groups and this resemblance is based on both a common environment and genetics. Thus differences in associations observed between mothers and daughters and mothers and sons may be a result of the exposure of the former to a common familial environment that is not experienced by mothers and sons.

Studies in monozygotic (MZ) and dizygotic (DZ) twins more precisely define the extent of heritability of bone mass. The difference between the intrapair variance of MZ (rMZ^2) compared with DZ (rDZ^2) twins provides a measure of heritability, termed an index. In this model it is assumed that the degree of shared environment is no different for DZ and MZ twins. Dequeker et al (1987) and Pocock et al (1987) reported a significantly higher correlation of bone density within adult MZ twins compared with adult DZ twins at the lumbar spine and estimated the heritability to be 0.85 to 0.88. Similar data were reported for the proximal femur and the forearm, suggesting that genetic factors contribute less at these

sites (heritability 0.75 to 0.78). Flicker et al (1992) reported higher correlation for total body bone mineral and lumbar spine and femoral neck BMD in MZ compared with DZ adolescent twins (excluding those over 45 years old), and estimated the heritability index to be 0.6 - 1.0 for adolescents and 0.4 - 0.6 for the elderly. Takeshita et al (1992) reported a considerable contribution from genetic factors to BMD at the lumbar spine and femoral neck in Japanese twins. From measurements of BMD at five sites in adult female twins, Christian et al (1990) estimated heritability indices of between 0.70 and 1.03 for BMD. They proposed that the high heritability indices suggested that the interaction of multiple genes, probably with multiple environmental factors, contributed to variations in spine and hip bone density. Slemenda et al (1991a), using the same data, questioned the assumption of the twin model that environmental influence is the same in MZ and DZ twins. They found this was not so in their analysis and concluded that heritability estimates were too high and that this may relate to a complex gene environment or gene-gene interaction.

Kelly et al (1990a) reported significantly greater rMZ (0.81) than rDZ (0.21) for serum osteocalcin, a measure of bone formation, and estimated that 80% of the variance in osteocalcin levels was explained by genetic factors. Tokita et al (1992) reported similar results for heritability indices of bone resorption (rMZ=0.72, rDZ=0.33). In a more recent study Kelly et al (1993) measured the rate of change in bone density at the lumbar spine and femoral neck in MZ and DZ twins. They showed there was a genetic effect on rate of change at the lumbar spine (rMZ=0.93, rDZ=0.51), although such an effect was not shown at the femoral neck. This evidence demonstrates that genetic factors contribute between 60-90% of the variance of peak bone mass, bone turnover, and age-related changes in bone mass in MZ twins, and the genetic effect appears to be stronger at the sites with higher proportions of trabecular bone, where bone turnover is greater, than at sites with predominantly cortical bone.

Bone density and other components of bone strength are complex phenotypes whose heritability is almost certainly polygenic. There are two approaches to mapping genetic markers of complex quantitative phenotypes. One is to examine phenotypes for linkage to candidate genes that are allelic, and the other is an association study in which the prevalence of

the alleles of the candidate gene in affected and nonaffected unrelated subjects are compared (Peacock 1995). The study by Morrison et al (1994) is both a linkage and association study. They identified a vitamin D receptor (VDR) gene and showed that VDR alleles contributed significantly to genetic variances in bone mass at the spine and hip, in twins and pre- and post-menopausal women. Common allelic variation in the vitamin D receptor gene was also shown to correlate with osteocalcin levels, suggesting a correlation with bone turnover (Morrison et al 1992).

A number of conflicting reports have since been published and these have been reviewed by Eisman (1995b) and Peacock (1995). Hustmeyer et al (1994) did not identify a VDR effect in their twin sample not did they find a VDR effect in their population sample. Whilst Garnero et al (1995) in a study of pre-menopausal women did not find significant differences in BMD between the two different homozygous genotypes, the differences observed were in the same direction as those reported by Morrison et al (1994). A study in Japanese women reported VDR allele related differences in BMD, but with allelic frequencies quite different from that in Caucasian women (Yamagata et al 1994). A Scandinavian study reported a non-significant trend to the relationship between osteoporosis and VDR alleles (Melhus et al 1994). Eisman (1995b) questioned whether some of the negative studies were a result of a type II error and also proposed that the differences between populations may reflect other genes moderating the effect of the VDR gene. An alternative explanation proposed was that differences in environmental factors, such as vitamin D and calcium, may permit or block the phenotypic expression of VDR gene allelic effects. He concluded that although there was little doubt that vitamin D receptor gene alleles modulate a significant proportion of the genetic factors regulating bone density, bone turnover and probably rates of bone growth and loss, the precise strength of the VDR gene effect is unknown and it is likely that other genes contribute to this genetic variance.

Peacock (1995) in reviewing these conflicting studies suggested that VDR alleles cannot account for the majority of the heritable component of bone density as Morrison et al (1994) proposed, nor are the alleles consistently associated with either the rate of bone loss or

turnover. However Peacock also indicated that due to the small sample size in many negative studies, VDR allele linkage to a small component of bone density cannot be excluded. To determine how many gene loci are in linkage with the phenotypes that determine bone strength, he suggested that more linkage studies are required in large numbers of related subjects, using short tandem repeat polymorphism distributed throughout the genome.

2.3.2 Nutrient intake

2.3.2.1 Calcium metabolism.

For calcium to be retained in the growing skeleton an individual must be in positive calcium balance. At any time of life calcium balance is a function of dietary intake, absorption of both dietary and digestive juice calcium, and urinary and faecal excretion and dermal losses. The daily calcium retention requirements for growth are: infancy 100 mg, childhood 90-100 mg, adolescence 90-210 mg (boys 180-210, girls 90-110). Bone consolidation between ages 20 and 30 years requires a total calcium retention of about 120 g, or 30 mg per day (American Academy of Paediatrics 1978).

There is no evidence that suggests that variations in the amount of dietary calcium affects the mineralization process as long as serum calcium levels are maintained, either during growth or later. Rather, the effect of a low calcium intake on the growing skeleton is confined to bone density, mediated through modulation of the balance between bone formation and bone resorption. While sufficient exogenous calcium must be present to sustain bone density during growth and to maintain skeletal mass later in life, additional calcium above this threshold will not produce more bone than is determined either by genetic influences or by current levels of mechanical loading (Heaney 1993b).

Matkovic (1991) analysed 487 calcium balance studies from published reports to assess calcium requirements and metabolism (absorption, retention and urinary excretion) during acquisition of peak bone mass, from infancy to young adulthood. Subjects were divided into four age groups, infants (2-12 months, mean age 0.5 years), children (2-8 years, mean 6 years),

adolescents (9-17 years, mean 12 years) and young adults (18-30 years, mean 22 years). A summary of this is shown in Table 2.4

Table 2.4
Components of calcium balance in different age groups (mean values) (Matkovic 1991)

Age range (mean)	Ca intake mg/day	Urine Ca mg/day	Ca balance mg/day	Net Ca absorption mg/day	%
2-12 mths (0.5)	1048	37	393	429	40
2-8 yrs (6)	1093	87	206	287	27
9-17 yrs (12)	1416	127	300	425	30
18-30 yrs (22)	695	154	-13	141	20

Urinary calcium rose with age from a mean low of 37 mg/day in infants to a mean maximum of 159 mg/day at 15 to 16 years. Mean calcium retention was very high in infants and adolescents (393 and 300 mg/day respectively) and lower in children (206 mg/day). Young adults were in slightly negative balance. Net calcium absorption was high during infancy (40%), declined in childhood (27%), increased again in adolescence (30%), and declined again during young adulthood (20%).

The main determinants of calcium balance in infants were vitamin D and dietary calcium. With the same amount of calcium in the diet, vitamin D supplemented (cod-liver oil) infants had substantially higher calcium retention (465 mg/day) than did non-vitamin D supplemented infants (280 mg/day). Infants were able to retain 400-500 mg calcium per day at intakes of approximately 1200 mg. Further increases in intake did not have a significant effect. Calcium excretion in the urine was very low (10-40 mg/day) and was not related to calcium intake. Infants could retain up to 44% of calcium intake.

Rate of growth slows during childhood and Matkovic (1991) described a parallel decline in calcium absorption and skeletal retention. He estimated that children (2-8 years) on an average intake of 1100 mg/day retained approximately 18% of intake, or 200 mg/day. Further increases in intake up to 1600 mg/day led to an increase in calcium balance of 304 mg/day whilst urinary calcium increased only by 45 mg/day. Urinary excretion of calcium was much higher in children (82 mg/day) than in infants. From multiple regression analysis calcium intake was identified as the most significant predictor of urinary calcium output. Intestinal calcium absorption was reduced in proportion to the lower calcium needs. Children had a similar calcium absorption (27%) as adults (20%).

Calcium metabolism in adolescents differed significantly from that in childhood and had some similarities with calcium metabolism during infancy. In general, adolescents retained more calcium than either children or young adults. However at very low intakes adolescents were in a lower positive calcium balance than children primarily because of greater urinary calcium loss. Calcium intake was one of the main determinants of calcium balance in adolescents; net calcium absorption increased with intake and urinary calcium did not change (Matkovic et al 1990). At each level of calcium intake, calcium balance in adolescents was substantially above the figures for young adults. A relatively high retention (369 mg/day) was recorded on a mean calcium intake of 1169 mg/day. Further increase in calcium intake reduced net absorption to 12% and increased faecal calcium excretion, whilst there was only a slight increase in calcium retention to 374 mg/day. This suggests that absorbed calcium of about 500 mg/day saturates the skeleton of adolescents which then indirectly leads to suppression of calcium absorption. Urinary calcium increased during the period of adolescence and reached its maximum by the age of 15-16 years or by the cessation of puberty. The main determinant of urinary calcium in adolescents was body weight, not calcium intake. In young adulthood (18-30 years) calcium absorption was substantially lower than in younger groups, and calcium excretion was the highest, so young adults needed more than 950 mg per day dietary calcium to reach positive calcium balance. The main determinant of urinary calcium in this age period was net calcium absorption.

From further analysis of these balance studies Matkovic and Heaney (1992) established the existence of an intake threshold for calcium ie the level of calcium intake below which skeletal accumulation of calcium varied with intake and above which it remained constant. Table 2.5 presents data from the regression analyses; this shows the threshold intake at which balance no longer rose with intake; the x-axis intercept (the intake at which balance is zero, a measure that accounts for absorption efficiency and minimum obligatory excretory losses through urine and faeces); and the slope of the regression line, which is a measure of the avidity of the skeleton for quantities of calcium ingested above the zero-balance intake. The threshold value varied with the stage of growth whilst the intake at which balance was zero increased with age from a low of 13 mg/day in infants to a high of 732 mg/day in young adults. The slope also varied with age. It was highest in infants and adolescents, less in children and least in young adults.

Table 2.5
Dietary calcium threshold intakes for age; levels of intake at which balance is zero; and the slope of the regression line (Matkovic and Heaney 1992).

Age years	Threshold mg/day	Intake at which balance zero mg/day	Slope
0-1	1090	13	+0.407
2-8	1390	183	+0.238
9-17	1480	320	+0.356
18-30	957	732	+0.200

Some authorities believe that the determination of calcium requirement as the intake at which balance is zero confounds and consequently predicts the asymptotic relationship between balance and intake because intake is included in both the x and y axis (Kanis and Passmore)1989). However the alternative method of determining calcium requirement, namely that

intake at which absorption and urinary excretion are equal, produces similar results (Nordin 1990).

In summary calcium balance varies between age groups according to calcium requirements. Calcium intake and skeletal modelling and turnover, determine calcium balance during growth. The highest requirements for calcium are during infancy and adolescence and then during childhood and young adulthood. To meet high calcium requirements infants and adolescents have higher absorption. Urinary calcium excretion increases with age reaching a maximum by the end of puberty. Because calcium intake directly influences skeletal retention, a low consumption of calcium in adolescence may limit longitudinal bone growth and influence adult height and/or predispose to decreased bone density. Adequate calcium intake during adolescence could therefore play an important role in the attainment of peak bone mass.

The current USA Recommended Dietary Allowances (RDA) (NRC 1989), the Australian Recommended Dietary Intakes (RDI) (NHMRC 1989) and the optimal calcium requirements recommended by the NIH Consensus conference on calcium intake (NIH 1994) are shown in Table 2.6. What is not known is the extent to which intake deficits in one phase of growth can be repaired later or how long the opportunity for such repair may last.

Table 2.6
Current USA RDA, Australian RDI and NIH optimal calcium intake .

Age years	USA	Age years	Australia		Age years	NIH
2-8	800	2-8	800		1-5	800
9-17	1200		boys	girls		
		8-11	800	900	6-10	800-1200
		12-15	1200	1000		
		16-18	1000	800	11-24	1200-1500
18-30	800	18-30	800			

2.3.2.2 Problems in dietary methodology

Methodological problems may account for the failure in some studies to find an association between calcium intake and bone status. Such problems include the assumption of stability of calcium intake over time, the lack of any single diet intake method being a precise measurement of usual intake, and that calcium intake is not a measure of calcium balance.

In many studies it is assumed that current calcium intake is the same as average lifetime intake or even remains constant over the time intervals between sequential bone mass measurements. Heaney et al (1990a) showed that even at intervals as short as six months current intake estimates accounted for only 40% of the variance of intakes at an earlier time, and over longer intervals the predictive value of one estimate in respect to a second decreased even further. Dietary intake in adolescents can be very erratic with large intra-individual variation (Bull 1988), and a tendency to under-estimation particularly in girls (Livingstone et al 1992, Magarey and Boulton 1994a). A longitudinal study of the dietary intake of 140-230 children in Adelaide showed that the correlation coefficient (tracking coefficient) for calcium intake expressed as both absolute intake (mg/day) and density (mg/MJ) was fairly stable for two year intervals with values of approximately 0.5. Thus 25% of the variance in calcium intake at a later age was accounted for by the value at the initial age. For intervals of four to five years slightly lower values for r were obtained, and for longer periods still lower values (Boulton et al 1995b, unpublished data). Tracking coefficients for absolute calcium intake in boys and girls from the Adelaide Nutrition Study (where $n>50$) and the present study (where $n<50$) are shown in Table 2.7.

Table 2.7

Tracking coefficients of mean calcium intakes (mg/day) in age intervals in boys and girls.

Initial age		Interval (years)									
years		2-3	n	4-5	n	6-7	n	11	n	13	n
4	boys	0.49	83	0.37	76	0.14^{ns}	71	0.12^{ns}	69	0.01^{ns}	44
	girls	0.54	62	0.56	61	0.28^{a}	56	0.20^{ns}	51	0.41^{b}	32
8	boys	0.68	70	0.59	66	0.48	67	0.49	43		
	girls	0.40^{b}	55	0.37^{b}	50	0.30^{a}	50	0.12^{ns}	33		
11	boys	0.45	121	0.55	116	0.46	41				
	girls	0.48	118	0.45	114	0.02^{ns}	37				
13	boys	0.47	118	0.63	41						
	girls	0.46	112	0.28^{a}	37						
15	boys	0.62	41								
	girls	0.30^{a}	36								

$p<0.001$ except ns: not significant, a: $p<0.05$, b: $p<0.01$

There is no single method of estimating dietary intake that can be a precise measurement of overall intake over a long period. Food records and recall are methods used to determine intake for specified days. However due to the magnitude of day-to-day variation within the same individual, intake measured on a particular day can be a very poor estimate of the average intake of that individual over a period of several weeks or months, and thus a poor measure of usual intake. Estimation of coefficients of variation from recording methods of assessing dietary intake from 15 population groups showed that variability from day to day is closely related to the nutrient being studied, and for energy ranged from 20 to 30% and for calcium from 30 to 50 % (Bingham 1987). Day to day variation may be even greater in adolescents particularly girls who may often have quite erratic and bizarre eating habits (Bull 1988). The diet-history and food frequency methods were designed to overcome the problem of day-to-day variation by asking what was the pattern of eating over a relatively extended period. A

review of dietary assessment methods by Bingham (1987) and a more recent report of the comparison of several methods used in the same subjects (Bingham et al 1994), concluded that unbiased retrospective estimates of diet are unobtainable and that short methods such as food frequency questionnaires are unable to fulfil the purpose for which they were designed, which is to categorize individuals into extremes of a population distribution. Beaton (1994), in analysing error in diet methodology concluded that the food frequency method had an error structure equal to that of three days of dietary data collected as records or as 24 hour recalls. He proposed that whilst food frequency methods reduced the real variance between subjects, they introduced new (spurious) between-subject variance. He concluded this may arise due to the inability to ask about all foods consumed and hence the questionnaire resulted in under-reporting of variation.

The problem of day-to-day variation in estimating true intake may in part be overcome by extending the number of days over which intake is recorded. Estimates of the number of days of records required to estimate individual intake within 10% of usual intake varied substantially between individuals for the same nutrient and within individuals for different nutrients (Basiotis et al 1987). Based on data from the year-long Beltsville dietary intake study which included 365 days of diet records from 13 male and 16 female adult subjects, Basiotis calculated that the mean (range) number of days required to estimate energy intake was for men 27 (14 to 84) and for women 35 (14 to 60) and the same figures for calcium were for men 74 (30 to 140) and for women 88 (35 to 168) days. Nelson et al (1989) determined the number of days required to rank individuals into tertiles, quartiles or quintiles with a given degree of accuracy. Accuracy of ranking can be expressed as the correlation coefficient (r) between the observed and true intakes. The calculation of the number of days is based on the ratio of within- to between-subject variances in nutrient intake. For children 4 to 17 years of age the number of days required to ensure $r>0.9$ for energy was 9 for boys and 10 for girls, and for calcium was 4 and 12 for boys and girls respectively.

Unfortunately diet record periods greater than 7 days are unlikely to be tolerated resulting in sample attrition. Selection of a 4-day period is often chosen as a compromise between

compliance and precision and this can be increased by repeated observations (Bingham 1987). The greater the number of repeated records the greater the precision.

Within any chosen method of dietary intake assessment there are a number of additional areas of potential error apart from that of day-to-day variation, namely the method of data collection, the accuracy of recording and the range of foods and the accuracy of the information of the data base.

Adequacy of calcium nutrition is more than just a matter of calcium intake. The critical factor which may influence the rate of bone accrual and thus final peak bone mass is calcium balance, and this does not necessarily equate to intake. Low calcium balance may result from excessive obligatory loss, low dietary intake or poor calcium absorbability. Loss and absorption vary with age. Urinary calcium rises through adolescence from childhood values of 80 mg/day to adult levels of 170 to 240 mg/day (Peacock 1991). Endogenous faecal calcium loss and dermal losses have not been estimated in children and adolescents but are probably related to body size (Peacock 1991). Weaver et al (1995) performed 3 week metabolic studies on 14 adolescent girls and 11 young women. They found that the adolescents had a lower urinary calcium excretion, a lower faecal calcium excretion, and a greater net absorption than the young women. A study by Abrams and Stuff (1994) reported that calcium absorption in girls varied with pubertal stage being highest in early puberty compared with pre- and late puberty periods. Heaney et al (1988a) estimated that there was a 6-7% variability in calcium absorption around any given absorption value within an individual human subject.

Calcium bio-availability and absorption are affected by the pH of the intestinal contents as well as a number of dietary components which increase (lactose) or decrease (phytates, oxalates, cellulose) these processes (Allen 1982, Heaney et al 1988b, Heaney and Weaver 1992). However Heaney et al (1990b) reported a significant association between calcium absorption fractions in women across differing dietary calcium loads, time intervals and between substances of differing intrinsic absorbability, in other words they found the individual's calcium absorption efficiency to be relatively consistent.

In theory dietary fibre might interfere with the bio-availability of co-ingested calcium by complexing with it, and although some studies have shown that certain fibres such as wheat bran do interfere with absorption, the effect over an intake range normally seen in children and adults is not very great. For example, an individual who doubles his/her wheat bran fibre intake from 10 to 20 g may expect a 6-10% decrease in food calcium availability (Heaney 1993b). Other fibres do not interfere at all.

Caffeine increases urinary excretion of calcium and therefore may affect calcium balance. In younger women with an adequate calcium intake caffeine may have little or no deleterious effect as increased urinary losses of calcium can be compensated for by an increased intestinal calcium absorption (Massey and Whiting 1993, Hansen 1994). In older women who are unable to compensate adequately caffeine may accelerate bone loss in those women whose daily calcium intake is below the recommended level (Hernandez-Avila et al 1993, Harris and Dawson-Hughes 1994).

Nonetheless in field studies in which dietary calcium intake is being measured, it is not possible to take into account the extent of influences on the absorption of calcium, namely the source of calcium in the diet and other nutrients in the diet, nor inter-subject variability in absorption and excretion.

2.3.2.3 Calcium intake and bone status: overview

The evidence for a relation between bone density and estimated calcium intake in individuals and populations is conflicting. The possible importance of nutritional factors to the development of PBM was shown by Matkovic's study in Yugoslavia (Matkovic et al 1979). On the assumption that hereditary and physical activity were the same in the two populations, he compared the bone mass of two populations, one with a high intake of dairy products and the other not. Bone mass measured at age 30 in each population revealed significantly greater values for men and women in the high calcium intake community.

A majority of published studies have described a significant positive correlation between calcium intake and bone mass, with r values ranging from 0.03 to 0.26. This is the magnitude that might be expected given the multitude of other factors known to affect bone mass. Meta-analysis of studies in premenopausal women with mean ages from 29 to 43 years found a persuasive case for a high calcium intake being associated with a higher BMD in mature and older adults (Cumming 1990). More recently Kelly et al (1990b) reported a strong correlation between calcium intake and vertebral bone mass in men, with calcium intake explaining 24% and 42% of the variance in bone mass at the lumbar spine and femoral neck respectively.

2.3.2.4 Studies in children and adolescents

Cross-sectional studies

Studies of the association between dietary calcium and bone mass in childhood and adolescence have produced conflicting results. Miller and Johnston (1990) reported that calcium intake was significantly correlated with bone mineral density in nine-year-old twins at the hip, spine and radius in males and the hip and spine in females, when height was controlled. Chan (1991) studied children aged 2 to 16 years and reported that the dietary calcium intake was positively associated with BMC. Children ingesting more than 1000 mg per day had higher BMC than those ingesting less. Turner et al (1992) measured BMD in 15 to 17-year-old girls and estimated that calcium intake contributed 5% and 9 % respectively to BMD variability at the femur and trochanter. Lee et al (1993) reported differences in radial BMD of children from two regions of differing calcium intakes. Sentipal et al (1991) in a cross-sectional study showed that calcium intake was one of three significant predictors of BMD. Maturational age (determined by Tanner Sexual Maturity Rating), chronological age, and calcium intake accounted for 80% of the variance in vertebral BMD.

Other studies have found no association. For example Katzman et al (1991) found no significant correlation of calcium intake with bone mass in 9 to 21-year-old females. Glastre et al (1990) found no correlation between BMD and calcium intake in children aged 1 to 15 years when controlling for age, and Nowson et al (1992) found no association with calcium intake and BMD at three sites in adolescents.

Differences in results from cross-sectional studies may be due to the problems associated with estimation of nutrient intake but also to the variability in calcium intake as estimation at one point is a poor indicator of intake over a longer period (Heaney et al 1990a). During adolescence when food intake is particularly variable (Bull 1988), nutrient intake estimated from a single assessment is likely to be very different from intake over a longer period. This problem may be partly overcome by assessing intake at a number of points. Such an approach will enable identification of those subjects who have an apparently consistently high or consistently low intake.

Longitudinal intervention studies

A number of researchers have studied the effect of calcium supplementation on bone mass, over periods of one to three years (Table 2.8). Most of these studies have demonstrated a positive effect of calcium on BMC and aBMD and in the study of Matkovic et al (1990) the authors proposed that the absence of significant differences in bone mass measurements between the supplemented and non-supplemented groups may have been due to a type II error where sample size was small.

Johnston et al (1992) conducted a three year double blind placebo-controlled trial of the effect of 1000 mg calcium citrate malate per day on bone mineral density in 45 pairs of twins. Among the 22 pairs who were pubertal throughout the study, those twins given supplements had significantly greater increases (between 1 and 5%) in aBMD at the mid-shaft and distal radius, hip and lumbar spine. Among the 23 pairs who went through puberty, or were post pubertal, there was no benefit to the supplemented twin. However one year after supplementation had ceased the significant difference in aBMD had disappeared (Slemenda et al 1993). In the year without supplement the placebo group had greater gains in aBMD at all sites. The authors proposed that calcium has its effect through suppression of remodelling and maintenance of the beneficial effects on gain in bone mass requires continued intakes of calcium at levels necessary to slow remodelling.

Table 2.8

Summary of intervention studies in children

Author, year	Subjects			Duration years	Site	Bone measure[a]	Calcium	Supplement	Outcome
	N control, supplemented	sex	age				Quantity mg/day	Form	
Matkovic et al 1990	8,20	f	14	2	proximal radius, distal radius	BMC BMD	1000	calcium carbonate or milk	not significant
Johnston et al 1992	45,45	m/f	6-14	3	proximal radius, distal radius, LS FN, prox femur	BMD	1000	calcium citrate malate	positive in pre-pubertal twins
Lloyd et al 1993	48,46	f	12	1.5	LS, TB	BMC BMD	500	calcium citrate malate	positive
Nowson et al 1994	32, 32	f	11-17	1	LS total hip	BMD	1000	lactate gluconate	positive
Andon et al 1994	62, 63, 63 b	f	10-13	0.5	TB	BMD	500,1000	calcium citrate malate	positive
Lee et al 1994	82, 80	m/f	7	1.5	distal radius	BMC BMD	300	calcium carbonate	positive
Gilchrist et al 1995	52, 53	f	15-16	1	hip,LS,TB	BMD	1200	dairy foods	positive
Chan et al 1995	24, 24	f	9-13	1	radius,LS,FN, TB	BMD BMC	700	dairy foods	positive in TB, LS only

a: BMD as areal BMD (aBMD) in all cases
b: 2 supplemented groups

Lloyd et al (1993) conducted a randomized, double-blind, placebo-controlled trial of the effect of 18 months calcium supplementation with 500 mg per day of calcium citrate malate on 94 girls, mean age 11.9 years. Increases in LS and TB aBMD of the supplemented group were 2.9% ($p=0.03$) and 1.3% ($p=0.05$) higher compared with the placebo group. In a study of 32 twin pairs aged 11 to 17 years, Nowson et al (1994) reported an increase in spine and hip aBMD after one year, in the twin given 1000 mg/day calcium. Andon et al (1994) assigned 248 healthy girls aged 10 to 13 years to a placebo group or one of two supplement groups (500 mg or 1000 mg per day of calcium citrate malate) for 6 months. Increases in TB aBMD were 1.0% and 2.2% greater in the intermediate and high supplement groups respectively compared with the control group. Lee et al (1994) conducted a randomized, double blind, controlled calcium supplementation (300 mg/day for 18 months) study on 162, 7-year-old Chinese children with habitually low calcium intakes (280 mg/day), a level considerably below the threshold value. They reported significantly greater gains in BMC ($p=0.02$) and aBMD ($p=0.008$) of the distal radius.

Two recent studies have used dairy foods as the source of calcium supplement. Gilchrist et al (1995) in a study in New Zealand reported significant increases in aBMD in a group of 15 to 16-year-old girls given a daily supplement of 1200 mg of calcium in the form of dairy products for 12 months, compared with the control group. A similar study over one year on 48 Tanner stage 2 girls by Chan et al (1995) reported that the subjects who supplemented their diet with two to three serves of dairy foods a day (equivalent to 700 mg calcium) had increases in TB BMC and LS aBMD that were 6.6% and 9.9% respectively, greater than the increases in the controls but there were no significant differences in radial or FN aBMD.

While earlier studies suggested that increases were more likely to occur in pre-pubertal subjects these latter two studies indicate that increases may also occur in pubertal girls but these increases are possibly site dependent. Similar studies have not been conducted in pubertal boys. In none of these studies however, has there been long-term follow-up measures to determine if these gains are maintained after supplementation ceases. It is possible that the effect of increased calcium is to suppress bone turnover and that the short duration of the

studies has allowed insufficient time for a new steady state to be reached. Additionally these studies have measured only BMC and aBMD which do not fully account for differences in bone size.

2.3.2.5 Studies in young adults and premenopausal women

Cross-sectional studies

Sowers et al (1985) reported that women whose estimated calcium intake from a food frequency questionnaire was greater than 800 mg per day had significantly greater bone mass than women whose intake was less than 800 mg per day. Similarly Tylavsky et al (1992) showed that radial BMC and aBMD in 18 to 22-year-old females was related to long-term calcium intake assessed as intake of milk and cheese. Hirota et al (1992) demonstrated that bone density correlated with dietary habit from infancy to the present in 19 to 25-year-old Asian women, and Fehily et al (1992) showed the same in Irish women. Kanders et al (1988) showed that vertebral and radial aBMD were significantly correlated with total calcium intake, but that the aBMD did not increase with daily calcium intakes greater than 800 to 1000 mg. Halioua and Anderson (1989) reported that women aged 20 to 25 years with an intermediate or high lifetime calcium intake (>500 mg/day) had significantly greater distal and midshaft radial BMC and aBMD when adjusted for physical activity. Metz et al (1993), in a study of 38, 24 to 28-year-old Caucasian women, showed by multiple linear regression that physical activity and calcium intake were positively associated, whereas protein and phosphorus were negatively associated with radial BMC and aBMD. A study by Kelly et al (1990b) on men found that dietary calcium was a significant predictor of aBMD of the axial bones but not of the forearm. Nowson et al (1992) found no association between calcium intake and bone density in three sites in young women and older pre-menopausal women.

The majority of these studies suggest a role for calcium intake in describing the variability in BMC and aBMD in young adults and that subjects with higher intakes have greater bone mass however the differences are relatively small and may in part be attributable to differences in bone size which cannot be accounted for when measuring aBMD.

Longitudinal observational and intervention studies (Table 2.9)

The results of two longitudinal observational studies in premenopausal women (Recker et al 1992, Mazess and Barden 1991) showed no association between the rate of gain of BMD and calcium intake, although in the study by Recker a positive association was found between rate of gain of spinal aBMD and the calcium to protein ratio of the diet. Three intervention studies have shown conflicting results. In a randomized study, Baran et al (1989) reported that there was no change in aBMD in the supplemented group over the 3-year period compared with a fall in aBMD in the control group to values significantly lower than the supplemented group. In the study by Smith et al (1989) on appendicular bone, no difference in bone loss between the supplemented and non-supplemented group was found. Similarly in the study by Friedlander et al (1995), which also included assignment of subjects to an exercise or stretching programme, there were no significant differences in changes in FN and trochanter aBMD between the supplemented and non-supplemented groups.

It may be that in children the effect of calcium intake above a certain amount is to accelerate bone growth to a fixed peak mass which is determined by genetic factors and mechanical loading. Thus those with a higher calcium intake reach their peak bone mass at an earlier age than those on a lower calcium intake but ultimately all subjects reach their genetically determined peak bone mass. None of the reported studies have measured BMD as a volumetric measure (vBMD). Bone status has been reported as BMC and aBMD both of which are dependent on bone size. vBMD which takes account of differences in bone growth will give a true indication of the effect of calcium on bone density. The establishment of an age-related threshold (the level of calcium intake below which skeletal accumulation of calcium varied with intake and above which it remained constant (Matkovic and Heaney 1992)), and the evidence from some studies in young adults, suggest that calcium has a role in permitting a higher PBM (Recker et al 1992) and in maintaining that value to menopause (Baran et al 1989).

Table 2.9
Summary of longitudinal and intervention studies in pre-menopausal women

Author	Subjects N	age	Duration years	Site	Bone measure[a]			Outcome
Longitudinal						Estimation of	calcium intake	
Recker et al 1992	156	18-26	1-5	LS	BMD	7-day diet diary each 6 mths		positive association Ca/protein ratio
Mazess and Barden 1991	240	20-39	2	FN, proximal femur, radius, humerus	BMD	quartiles of intake averaged from 0, 1 and 2 y		no association
Intervention						Calcium mg/day	Supplement form	
Baran et al 1989	17, 20	30-42	3	LS	BMD	610	as dairy products	no bone loss in supplemented group
Smith et al 1989	19,16	34-52	4	ulna, radius, humerus	BMC, BW, BMD	1500	calcium carbonate	no difference in bone loss
Friedlander et al 1995	29, 34	20-35	2	FN, trochanter	BMD	1500 including diet	calcium carbonate	no difference in bone changes

a: BMD as areal BMD (aBMD) in all cases

Calcium intake is therefore important throughout life. In infancy and childhood adequate calcium is required to ensure complete mineralisation of the growing bone. In late childhood, in the prepubescent years, calcium may have a role in facilitating the achievement of a higher peak bone status and intakes at least at the threshold level are recommended. However longitudinal studies, including the estimation of vBMD, are required to demonstrate the benefits of high calcium intakes in growing children. In premenopausal adulthood calcium has been shown in some studies to increase peak bone mass or prevent bone loss. Calcium is readily available from food and in this form should be encouraged throughout life. Dietary habits are often established at a young age and this may particularly apply to the consumption of dairy products.

2.3.2.6 Other dietary factors

A number of other dietary factors are important in the development and maintenance of bone. Vitamin D is essential for the absorption of calcium from the diet by facilitating active transport. This is particularly important for adaptation to low intakes. Thus vitamin D status can influence absorptive performance and hence effective calcium requirement (Heaney 1993b).

Phosphorus as phosphate makes up approximately half the weight of bone mineral and hence must be present in adequate quantities in the diet to mineralize and maintain the skeleton. There has been some concern that excessive phosphorus intake may be harmful as it transiently depresses ionized calcium levels and thus leads to increased secretion of parathyroid hormone (PTH) which could influence bone resorption. However the effect is transient. Under steady state conditions, an increased phosphorus intake reduces urinary calcium loss and increases digestive juice secretion of calcium. The two effects are approximately equal in magnitude hence total body calcium balance tends to be unaffected. An increase in phosphorus intake suppresses renal synthesis of calcitriol which could lead to decreased calcium absorption but at the same time urinary calcium losses are also suppressed (Heaney 1993c).

Energy and protein must be adequate to ensure growth, and deficiency in these will result in growth retardation. Adequate protein intake is also important for bone health in the elderly. BMD has been reported to be positively correlated with protein intake in elderly Caucasian and Asian women, and protein intake was inversely correlated with bone loss in older women (Heaney 1993b). Protein in excess however, increases urinary calcium loss due to the increase in acid load affecting tubular resorption of calcium, and thus interferes with calcium conservation in response to a restricted calcium intake. Doubling of protein intake results in an increase in urine calcium of about 50% (Margen et al 1974, Heaney and Recker 1982). At a high calcium intake the effect of protein can be offset by improved absorption efficiency; however at a low calcium intake the degree of adjustment possible is usually not sufficient to compensate for the protein-induced calcium loss (Heaney 1993c). Sodium increases urine calcium by competing with it for reabsorption in the renal tubules. This results in an increase in urinary calcium of 1 mmol for every 100 mmol increment of ingested sodium (Nordin and Polley 1987). The effect seems to be more marked at low calcium intakes.

Vitamin K is important in the synthesis of bone proteins and trace elements zinc, manganese, and copper are essential metallic cofactors involved in synthesis of various bone matrix constituents.

2.3.3 Physical activity

2.3.3.1 Physical activity and bone

Under normal circumstances there is a close match between the structure of bone with its particular function. Mechanical loads resulting from activity produce strains within bones which were first recognized to provide the stimulus for functional adaptation of the tissue by Heuter (1862) and Volkman (1862). This is clearly illustrated by a variety of congenital and acquired conditions in which failure of growth occurs in the appendicular skeleton e.g. long bones in a limb affected by polio or legs in a child with spina bifida, or the axial skeleton e.g. the mandible in conditions in which chewing does not occur. Weight or load bearing exercise therefore has a major role in determining and maintaining bone mass. Immobilisation and

micro-gravity result in loss of bone mass whilst those participating at high levels of activity in a wide range of sports have greater bone mass than more sedentary controls (Forwood and Burr 1993). However the threshold levels of activity associated with either positive or negative changes are poorly defined because of the complexity of the loading history inherent in a wide range of activities and the possible interactions with respect to age, physical and physiological status of an individual, and in particular the difference in response between growing and mature bone.

The physical and histological changes consequent on exercise on growing bone have been studied in animals and reviewed by Forwood and Burr (1993). In this model growing bone responds to low or moderate exercise through significant additions of new bone. This occurs in both cortical and trabecular moieties and these adaptations are maintained into and throughout adulthood. However there is a threshold level of activity above which limb bones respond by suppressing normal growth and modelling activity. This reduces geometric (bone length and volume), mechanical (eg load and displacement at maximum and failure points, flexural rigidity), and histological (number of osteocytes and osteoclasts, and osteous per unit area, porosity) properties in both cortical and trabecular bone (Li et al 1991).

These animal studies have shown that growing bone has a greater capacity to add new bone to the skeleton than the bone of adults. Growing and mature bone adopt different strategies for adaptation. Growing bone has a greater potential for periosteal expansion than mature bone allowing it to adapt more rapidly and efficiently to an acute need for increased strength, whilst mature bone increases bone mass by increasing osteonal mean wall thickness. There is no physiological explanation to explain the different response of young and old bone to physical activity but it may be related to the mechanical stimulus to remodelling that occurs in the growth plate and subperiosteal cambium cells.

Steinhaus (1933) and more recently and formally Frost (1990), postulated that optimum levels of pressure upon epiphyses produced by exercise stimulate bone until length is achieved. If pressure continues beyond this optimum, bone growth is retarded. Carter and associates have

developed models to predict the response of living bones to changes in mechanical loading but these require validation from experimental studies. In one study in which a model was applied to two running studies, stress magnitudes (or joint forces) had a greater influence on bone mass than the number of loading cycles (the frequency of application of the load)(Whalen et al 1988) .

2.3.3.2 Problems in physical activity methodology

In assessing physical activity and its potential effect on bone mass it is not possible to determine the relative stress magnitudes or the number of loading cycles of the wide range of activities in which normal individuals engage. Various methods have been used to assess physical activity but at best they can only broadly categorise subjects into high, medium or low activity levels with respect to effort expended, rather than stress magnitude. In addition there is a degree of uncertainty as to whether strain applied at a particular site can also have an effect on bone metabolism at a distant site. Thus any physical activity score is a crude estimate of the stimulus being applied to the skeleton or to a particular bone.

Standardized approaches to activity assessment in children have rarely been attempted (Slemenda et al 1991b). Studies in children and adolescents reporting the relationship between physical activity and bone mass have used a wide variety of methods to quantify physical activity: hours per week of walking and sporting activities in the previous year (Nowson et al 1992); a score based on the energy expenditure of exercise at home, school and in leisure time (Turner et al 1992); total hours of weight-bearing exercise determined from participation in defined activities such as physical education classes and team sports (Slemenda et al 1991b); and a categorisation into high, medium and low activity levels determined by the time spent in sporting activities (Katzman et al 1991, Kroger et al 1993). Other studies have selected elite or specialist athletes and compared these with an age-matched control group e.g figure skaters (Slemenda and Johnston 1993), cyclists (Rico et al 1993a) and gymnasts (Bass et al 1995). Muscle strength has also been used as a measure of activity and related to bone mass particularly in longitudinal and intervention studies.

Petrie et al (1993) assessed the association between each of aerobic capacity and the physical activity which reflected weight-bearing activity, and the muscle strength of the axial and appendicular skeleton in 96 women aged 29 to 40 years. They found that muscle strength was significantly correlated between axial and appendicular sites and concluded that axial musculature represents overall muscle strength. Aerobic capacity (maximal oxygen uptake) was not correlated with muscle strength. Physical activity score, a measure of weight-bearing activity, was significantly correlated with back extensor strength but not with aerobic capacity. They concluded that aerobic capacity was an invalid marker for level of daily weight-bearing physical activity.

2.3.3.3 Physical activity and bone status: overview

Much of the evidence cited for positive effects of exercise on the adult skeleton arises from cross-sectional studies. These generally demonstrate that individuals with a history of physical activity have greater bone mass than their sedentary counterparts. The magnitude of these differences is quite variable and depends on the type and intensity of the physical activity, the site measured, gender and the physiological stress of the individual, but are generally less than 10% (Forwood and Burr 1993). There are a number of methodological problems associated with many of these studies. Apart from the intrinsic error of the measures used, and the possibility of a type II error from inadequate power due to sample sizes, they do not account for exercise history (Forwood and Burr 1993) or selection bias (Gutin and Kasper 1992).

Studies in normal healthy individuals over a wide age range have found that more physically fit and stronger individuals have greater bone mineral density at axial and appendicular sites, and that this effect cannot be explained by genetic influence on muscle bulk. Physical loading at the extremes of stress has a major effect on bone structure, although such levels of loading may have little relevance to the effect of physical activity in normal individuals (Eisman et al 1991).

Studies of female athletes from different sports suggest that weight bearing exercises increase bone mineral density, and that the response of BMD to training is site specific (Kirk et al 1989, Davee et al 1990, Heinonen et al 1993). However excess physical activity in women, leading

to oestrogen deficiency, can have adverse effects on bone mass (Carbon 1992, Rutherford 1993). A study by Rico et al (1993a) of post-pubertal male cyclists found no difference in total body bone mineral compared with sedentary boys suggesting that cycling does not stimulate bone mass in young men. Although cross-sectional studies cannot test the hypothesis that exercise adds bone to the adult skeleton, and accepting the problems in interpretation from measurement and sampling bias, the overall conclusion is that weight-bearing exercise positively influences the attainment of strong bones as measured by bone density.

2.3.3.4 Studies in children and adolescents.

Studies in children and adolescents have mostly been restricted to cross-sectional studies and have reported variable associations between bone mass and physical activity. Nowson et al (1992) reported that physical activity in the previous year was not a significant covariate in determining BMD at three sites in adolescents. Turner et al (1992) estimated that 4% and 5% of the variance in BMD of the FN and trochanter respectively was accounted for by physical activity in 15 to 17-year-old females. Slemenda et al (1991b) reported that weight-bearing activity was positively associated with bone mass at the radius and hip in children aged 6 to 14 years even when controlling for age and sex, and high performance female figure skaters had greater than expected pelvic and femoral bone density, although there was no difference in the arms and spine (Slemenda and Johnston 1993). Conversely Katzman et al (1991) in a cross-sectional study of 9 to 21-year-old girls in which subjects were classified as having a high, moderate or low level of activity according to the number of exercise sessions per week lasting a minimum of 30 minutes, found no correlation between activity level and BMD.

There are no reports of physical activity intervention studies in children and adolescents. However in the study by Kroger et al (1993) of 7 to 20-year-old subjects in which changes in aBMD at the LS and FN were measured over a one year period, subjects were stratified according to their physical activity. In these subjects there was no significant relationship between the incremental increase of bone density and physical activity. Bass et al (1995) postulated that the timing of exercise might be an important determinant of bone growth. They studied pre-pubertal elite female gymnasts aged 7.5 to 14.3 years (mean age 10.3, mean bone age 8.9 years) and a control group of girls matched by bone age. The gymnasts had almost a

50% increase in BMC over a 6-month period, and a 190% increase in BMC as g/cm length but had reduced stature. They concluded that the pre-pubertal years may be the most opportune time to increase bone density through physical activity but that this may result in shorter stature. A prospective one year study of 470 boys and girls aged 8 to 16 years found that weight-bearing physical activity was a significant predictor of change in BMD of both the distal and ultradistal forearm in girls less than 11 years of age and in boys both older and younger than 11 years (Gunnes and Lehmann 1996).

Extreme levels of activity resulting in menstrual disturbance and athletic amenorrhoea can result in a loss of bone density (Carbon 1992, Rutherford 1993).

2.3.3.5 Studies in young adults

Cross-sectional studies

A number of authors have reported a positive association in young adult (premenopausal) women aged 20 years and older, between radial BMC and BMD and moderate physical activity (Metz et al 1993), long-term or lifetime activity (Halioua and Anderson 1989, Tylavsky et al 1992, Hirota et al 1992), and sports activity in adolescence (Fehily et al 1992) and childhood (McCulloch et al 1990). Kanders et al (1988) reported a similar association between vertebral BMD and current activity pattern.

Snow-Harter et al (1990), in a study of 18 to 30-year-old women whose activity levels varied from sedentary to active, concluded that muscle strength was an independent predictor of BMD and accounted for 15-20% of the total variance in bone density. In a second study in men (Snow-Harter et al 1992b), they found BMD of lumbar spine, femoral neck, right tibia, and total body to be significantly greater in exercisers compared with non-exercisers, and that muscle strength made an important contribution to the variance of BMD. Valimaki et al (1994) reported that regular exercise over the preceding 11 years was an important determinant of bone mass in subjects aged 20 to 29 years, in the femoral neck for both sexes and in the lumbar spine for men.

Such cross-sectional studies can be confounded by selection bias as it is likely that people who regularly participate in certain types of sporting and recreactional exercise have a temperamental and physiological, as well as a morphological pre-disposition to those activities. For example people may engage in strength training partially because they already are stronger than their peer group, and hence may already have a greater bone density (Gutin and Kasper 1992). Therefore only prospective studies are able to test the hypotheses with respect to the role of exercise in determining bone density.

Longitudinal observational and intervention studies

Mazess and Barden (1991) found no effect of daily activity on appendicular or axial BMD in 20 to 39-year-old women over a 2 year period, but Recker et al (1992) in a 5 year longitudinal study of 21-year-old women found the rate of total body bone gain was positively associated with physical activity. They concluded that PBM could therefore be enhanced by physical activity.

As with calcium intake and PBM, we can ask the question: does physical activity in youth hasten bone growth to a fixed peak mass which is determined by genetic factors or can exercise enhance the genetically determined PBM? Several longitudinal studies in young adults provide evidence that exercise can add small amounts to bone mass. A study by Williams et al (1984) measured BMC in the calcaneous of 20 male runners aged 38 to 68 years, before and after nine months of training. Subjects who ran consistently showed a significant increase in BMC of 3.1%. Runners who exercised inconsistently showed no increase.

Two intervention studies have reported positive effects of exercise on BMD. Snow-Harter et al (1992a) conducted a randomized exercise intervention trial over eight months in 52, 20-year-old women Thirty-one women completed the study of progressive training in jogging and weight lifting. Weight training significantly increased muscle strength and there were modest increases in lumbar BMD in runners (1.3-1.6%) and weight lifters (1.2-1.8%). These were both significantly different from controls. There were no significant changes in BMD of the

proximal femur. Friedlander et al (1995) conducted a 2-year study in young women aged 20 to 35 years. Thirty-two subjects completed a programme of aerobics and weight training and 31 participated in a stretching programme. There were significant positive differences in BMD between the exercise group and the stretching group of 2.3 to 2.5% for the LS and FN.

Physical activity has a role in maintaining bone health and reducing the risk of fracture throughout life. Physical activity is required for normal growth to ensure mechanical loading of the skeleton. Physical activity in the pre-pubertal years may increase bone density but in adolescence, physical activity at extreme levels which leads to exercise induced amenorrhoea, should be avoided. Studies in pre-menopausal women indicate that exercise can increase BMD and thus contribute to the prevention of osteoporosis. What has not been established is exactly how much exercise and the range of exercise that may be beneficial. As with dietary calcium intake, if exercise is a lifestyle habit in childhood it is more likely to be continued in adolescence and into adulthood. A more active adult may have a greater bone density than their less active counterpart, but they will also have greater stability and flexibility and thus be less likely to fall and hence sustain a fracture. The prevention of fractures is the ultimate goal in osteoporosis prevention.

2.3.4 Summary

Studies of bone mass between ethnic groups, family members of two or more generations and twins, have demonstrated a genetic component to the variability of PBM. Identification of a gene linked to at least a small component of bone density supports these findings. Estimates of heritability for bone density, determined from comparison of MZ and DZ twins have ranged from 0.50 to 0.80. Adequate calcium is required during growth to ensure complete mineralisation of the growing bone. Studies of an association between calcium intake and bone mass in children and adolescents have produced conflicting results. These differences may be attributed in part to the problems of estimating nutrient intake and that calcium intake is a very poor measure of calcium retention. Intervention studies in children and adolescents have shown that subjects taking calcium supplements for up to one year have greater increases in

bone mass and density compared with unsupplemented controls. However what is unknown is whether these greater increases are maintained following withdrawal of supplements, thus resulting in higher PBM in early adulthood. Physical activity is important to maintain bone health throughout life and is required for normal growth to ensure mechanical loading of the skeleton. Cross-sectional studies in children and adolescents have reported conflicting results with respect to an association between physical activity and bone mass. As with calcium, the problems with assessment of physical activity and the unknown relationship between various activities and the stress they produce on bone may explain these varying results. Studies of elite athletes suggest that physical activity can enhance bone mass in pre-pubertal children and studies in young adults suggest a role for physical activity in promoting modest increases in bone density.

Thus while much of the variance in bone mass/density is determined by genetic factors the modifiable lifestyle factors of nutrition and physical activity have a role in optimising that genetic potential and possibly enhancing it.

2.4 MEASUREMENT OF BONE STATUS

Techniques used to study bone accrual during childhood and adolescence include single photon absorptiometry (SPA), dual photon absorptiometry (DPA), dual energy x-ray absorptiometry (DXA), quantitative computed topography (QCT), and one study using quantitated roentgen microdensitometry (QMD).

SPA uses an iodine-125 source to produce a beam of collimated photons that pass to a multiplier detector. The forearm is positioned perpendicularly to the scan path and a measurement of bone mineral content (g/cm) is produced. By use of an edge detector the width of the bone can also be determined and an areal BMD (aBMD)(g/cm^2) calculated. Estimation of bone volume (Nordin et al 1986) enables determination of volumetric bone mineral density (vBMD) and eliminates differences in bone size. Application of fat correction to estimation of bone mass decreases possible sources of error, particularly when fat tissue is

changing over the period of a longitudinal study (Need et al 1989). An additional advantage of SPA is that the radiation dose is low. The surface dose is 30 to 50 uSv equivalent to an effective dose of less than 1 uSV (Genant et al 1991). SPA is also relatively low cost, easy to operate, causes little inconvenience for the subject and has very good precision and accuracy of 2-5% (Shaw and Bishop 1995). The disadvantage of SPA is that it can only measure peripheral bones which are principally cortical bone, due to the requirement of immersion in a water bath.

DPA uses two separate energy levels and produces a measurement of bone mineral content (g) and bone mineral density (g/cm^2) which are a reflection of the attenuation of the beam over the scanned area. DPA has a slightly higher radiation than SPA (surface dose 30-50 uSv, effective dose 5 uSv) but permits measurement of the axial skeleton (Genant et al 1991). Determination of axial bone mass is a more sensitive measure of trabecular bone (De Schepper et al 1991). Bone density measurements are as areal density (aBMD) and therefore do not take account of the three-dimensional size of the bones. DPA has an accuracy of 4-10% and a precision of 2-4% (Shaw and Bishop 1995). The point of bone mass estimation for repeat measurements in growing subjects can be more easily located at the spine than in the forearm however DPA does not take account of the diametrical growth of the bone.

DXA uses a beam of collimated x-rays from a source providing alternating pulses of 70 and 140 kV. DXA requires a minimal radiation exposure and a short scanning time (surface dose 10-30 uSv, effective dose 1 uSv) (Genant et al 1991). It has high accuracy and precision but is more expensive. It gives an integral measurement of trabecular and cortical bone density of the whole vertebra by correcting the bone mineral content (g) for the projected area of the bone. It does not correct for the anterior-posterior depth of the vertebra and thus the data are not a true density but an areal density (aBMD) and are influenced by the size of the vertebra. The bone density of small bones will be underestimated and that of large bones will be overestimated. DXA is the preferred method for estimation of spinal bone mass (Glastre et al 1990). It has an accuracy and precision of 1-2% (Shaw and Bishop 1995). DXA may also be used to estimate total lean body and fat mass.

QCT can measure true 3-dimensional density (vBMD) (Genant et al 1991). It can measure trabecular, cortical and integral (trabecular + cortical) bone at any site centrally or peripherally. It has a high cost and high radiation (surface dose 2 000 uSv, effective dose 60 uSv) and is thus not suitable for sequential measurements.

QMD has been used by only one author to determine true volumetric density of the digit (Trouerbach 1991) The procedure requires standard postero-anterior radiographs of the hand and a supplementary radiograph of the index finger. From the average of the attenuation coefficients of all the material present, bone mineral density is determined as mm Al equivalent/$mm^3 10^3$.

Longitudinal measurements of bone density will be confounded by changes in bone size if areal density rather than volumetric density is determined and the former is the case in the majority of studies. Several authors have attempted to overcome this problem. Nordin and Polley (1987) estimated the cross-sectional area of the radius and ulna from the width of the bones and thus derived a volumetric bone density. Katzman et al (1991) estimated bone volume of the spine, femur and total body using densitometry derived area and other skeletal length measurements and thus determined a true bone density measure. The derivation of these estimations has been described by Carter et al (1992). Lu et al (1995) assumed the mid-femoral shaft to be a cylinder and thus estimated volumetric bone density of this site.

In studies of adults both Nieves et al (1992) and Nelson et al (1992) reported SPA and DXA measurements on the forearm to be strongly correlated for aBMD r = 0.6-0.9, r =0.97 respectively. Karlsson et al (1993) found good correlation between SPA measurements of the wrist and DXA measurements of the total body (r = 0.9). Nilas et al (1987) measured total body and spinal bone mass using DPA, and forearm bone mass using SPA in normal women and those with vertebral fractures. They reported spinal bone mass was significantly related to forearm measurements (r= 0.6) and that the subjects with vertebral fractures had low bone mass at all sites. In contrast Mazess and Barden (1990) found lower correlations between sites

in young females suggesting that single site measurements may not be useful in studies in adults.

Miller et al (1991) measured BMC and BMD in the forearm using SPA, and in the hip and spine using DXA in 140 children aged 5 to 14 years. They reported a high correlation for bone mineral among bone sites e.g spine : radius, BMC r= 0.78 boys, 0.85 girls, BMD r= 0.69 boys, 0.89 girls. Even though the relative amounts of cortical and trabecular bone are different between radial and other sites they did not find the pattern of prediction equations using frame size and fatness different between sites. Katzman et al (1991) compared DPA and DXA measurements of the spine and whole body in 45, 9 to 21-year-olds. They found the measurements by the two methods to be highly correlated: spine r = 0.97, whole body r = 0.93. Importantly these observations indicate that measurement at one site is a good indication of bone mass at other skeletal sites, and that SPA measurements can be calibrated to DXA measurements.

2.5 ASSESSMENT OF BONE STATUS IN CHILDREN AND ADOLESCENTS

Tables 2.11 to 2.14 summarise cross-sectional studies describing bone mineral content (BMC) and bone mineral density (BMD) throughout childhood and adolescence. Studies have been classified according to the method of measurement of bone mineral namely SPA, DPA, DXA and QCT. Studies using SPA measure the appendicular skeleton, usually the radius, and report results as BMC in g/cm and areal BMD (aBMD) in g/cm^2. Studies using DPA, which principally measure the axial skeleton, in particular the lumbar spine, may report BMC as g but mainly report aBMD as g/cm^2. Studies using DXA usually make measurements of the spine but may also estimate total body bone mineral (TBBM) and appendicular regions. Results are reported as BMC in g and aBMD in g/cm^2. QCT is used to measure spinal bone density. Results are reported as true volumetric density, vBMD in g/cm^3. The measure of bone reported in each study is indicated in the tables.

These cross-sectional studies provide information on bone mass from a wide range of countries. It is only recently that there have been reports on Australian subjects. Lu et al (1994) have reported aBMD of TB, spine and femur on 266 males and females aged 4 to 27 years, and Henderson et al (1995) reported BMD and aBMD of spine, lower leg and femur of 115 18-year-old females. A second study by Lu et al has reported vBMD of FS in 204 boys and girls aged 8 to 27 years (Lu et al 1995).

Several longitudinal studies have also been reported and these are summarised in Table 2.14. All these studies have been of two years duration or less. No longitudinal studies have been reported on Australian subjects apart from a small sub-set of 55 in the study by Lu et al (1994) who had a second measure one year after the first. When the present study commenced in 1986 only four studies of bone mass estimation in children had been published (Christiansen et al 1975a, 1975b, Krabbe et al 1979, Landin and Nilsson 1981, Hui et al 1985), and only one longitudinal study on a group of 11 to 13-year-old boys (Krabbe and Christiansen 1984). In the 1990s with developments in bone density technology there has been a wealth of studies published, but there have been no previous longitudinal studies including males and females, and the age span of puberty.

Table 2.10

Bone density studies in children and adolescents using single photon absorptiometry (SPA).

Study	Year	Site	Bone Measure[a]	N	Age	Sex	Country	Ethnicity
Christiansen et al	1975a,b	radius	C, TB Ca	301	7-20	m, f	Denmark	
Krabbe et al	1979	radius	C	301	7-20	m, f	Denmark	
Landin and Nilsson	1981	forearm	C, aD, vD	131	4-16	m, f	Sweden	
Hui et al	1985	radius	aD	1225	6-19	m, f	USA	Caucasian
Dhuper et al	1990	radius	aD	43	13-20	f	USA	White
Matkovic et al	1990	radius	C, aD	40	14	f	USA	Caucasian
Chan	1991	radius	C	164	2-16	m, f	USA	White
Geusens et al	1991	radius	C, W, aD	202	3-25	m, f	Belgium	
Miller et al	1991	radius	C, aD	140	5-14	m, f	USA	White
Bishop et al	1992	radius	C, W	420	4-10	m, f	Britain	Caucasian
Johnston et al	1992	radius	C, aD	90	6-14	m, f	USA	White
Rubin et al	1993	radius	aD	299	6-18	m, f	USA	White
Lee et al	1993	radius	C	243	5	m, f	China,HongKong	Chinese
Gunnes	1994	forearm	C, aD	494	8-17	m, f	Norway	

a: C = BMC, W = bone width, aD = aBMD, vD = vBMD

Table 2.11
Studies in children and adolescents using dual photon absorptiometry (DPA)

Study	Year	Site	Bone measure[a]	N	Age of subjects	Sex	Country	Ethnicity
Dhuper et al	1990	spine, foot	aD	43	13-20	f	USA	White
Matkovic et al	1990	spine	C, aD	40	14	f	USA	
Ponder et al	1990	spine	aD	184	5-12	m, f	USA	White, Black, Hispanic
De Schepper et al	1991	spine	C, aD	136	1-18	m, f	Belgium	
Geusens et al	1991	spine	C, aD	202	3-25	m, f	Belgium	
Gordon et al	1991	spine	C, aD	236	3-30	m, f	Canada	
McCormick et al	1991	spine	aD	335	5-19	m, f	USA	White, Black, Hispanic
Miller et al	1991	spine, hip	C, aD	140	5-14	m, f	USA	White
Sentipal et al	1991	spine	aD	49	8-18	f	USA	Caucasian
Thomas et al	1991	spine, femur	C, aD	109	3-20	m, f	USA	Black, White
Grimston et al	1992	spine, femur	aD	74	9-16	m, f	Canada	
Johnston et al	1992	spine, femur	C aD	90	6-14	m, f	USA	White
Gordon and Webber	1993	TB, head, legs	C, aD	76	8-26	f	Canada	
Rubin et al	1993	spine	aD	299	6-18	m, f	USA	White

a: C = BMC, A = bone area, aD = aBMD, vD = vBMD

Table 2.12 Studies in children and adolescents using dual X-ray absorptiometry (DXA)

Study	Year	Site	Bone measure[a]	N	Age of subjects	Sex	Country	Ethnicity
Glastre et al	1990	spine	aD	135	1-15	m, f	France	
Bonjour et al	1991	spine, femur	C, aD	207	9-18	m, f	Switzerland	Caucasian
Henderson	1991	spine, femur	aD	20	4-15	m, f	USA	
Katzman et al	1991	TB, spine, femur	C, aD, vD	45	9-21	f	USA	
Southard et al	1991	spine	aD	218	1-19	m, f	USA	Mixed
Kroger et al	1992	spine, femur	aD, vD	84	6-19	m, f	Finland	
Lloyd et al	1992	TB	C, aD	120	11-13	f	USA	Caucasian
Tsukahara et al	1992	spine, femur, radius	aD	32	3-18	m, f	Japan	Japanese
Turner et al	1992	spine, hip	aD	138	15-17	f	New Zealand	White,Asian,Polynesian
Faulkner et al	1993	TB, regional	C, aD	234	8-16	m, f	Canada	
Lloyd et al	1993	TB, spine	C, aD	94	11-13	f	USA	Caucasian
Rico et al	1993c	TB	C, aD	154	5-18	m, f	Spain	
Rico et al	1993a	TB	C, aD	49	15-19	m	Spain	
Davie, Haddaway	1994	spine, femur	C, aD	132	5-13	m, f	Britain	White
Moreira-Andres et al	1994	spine, radius	aD	121	3-18	m, f	Spain	
Nishiyama et al	1994	spine	aD	342	6-15	m, f	Japan	
Lu et al	1994	TB, spine, femur	aD	266	4-27	m, f	Australia	
Lu et al	1995	FS	vD	204	8-27	m, f	Australia	
Henderson et al	1995	spine, femur, lower leg	C, aD	115	18	f	Australia	
Teegarden et al	1995	TB	C, aD	247	11-32	f	USA	Caucasian

a: C = BMC, aD = aBMD, vD = vBMD

Table 2.13

Studies in children and adolescents using quantitative computed topography (QCT) and quantitative rontgen microdensitometry (QMD).

Study	Year	Method	Site	Bone measure[a]	N	Age of subjects	Sex	Country	Ethnicity
Gilsanz et al	1988b	QCT	spine	vD	103	2-18	m, f	USA	White
Gilsanz et al	1991		spine	vD	150	2-20	m, f	USA	Black, White
Gilsanz et al	1994		spine	vD	196	4-20	m, f	USA	White
Mora et al	1994		spine	vD	96	4-20	f	USA	White
Trouerbach et al	1991	QMD	digit	vD	1190	7-11	m, f	Netherlands	White

a: vD = vBMD

Table 2.14

Longitudinal studies in children and adolescents

Study	Year	Method	Site	Bone measure[a]	N	Age (years)	Sex	Duration (years)	Country
Krabbe, Christiansen	1984	SPA	radius	C	36	11-13	m	2	Denmark
Goslings et al	1995	SPA	radius	C, W	392	6-12	m, f	2	Britain
Katzman et al	1991	DXA	TB, spine, femur	C, aD, vD	12	13-17	f	1.5 - 2.1	USA
Kroger et al	1993	DXA	LS, FN	aD, vD	28, 37	7-20	m,f	1	Finland
Theintz et al	1992	DXA	LS, FN, FS	C, aD	100, 98	9-19	m,f	1	Theintz
Lu et al	1994	DXA	TB, LS	aD	25, 28	4-17	m,f	1	Australia

a: C = BMC, aD = aBMD, vD = vBMD

2.6 BONE STATUS, GROWTH AND MATURATION

Through childhood until the pubertal growth spurt there is no significant difference in stature between boys and girls at the same stage of skeletal maturity. At puberty sexual dimorphism is accentuated although it is least in humans of all the primates. In girls the earliest influence from puberty on linear growth occurs at a median age of 10 years. The rate of growth then increases steadily from 5.5 cm/year until it reaches a median of 8 cm/year at the point of peak height velocity, which occurs at a median age of 11.5 in the US (Tanner and Davies 1985), and 6 months later in British children (Pan and Radcliffe 1992). Menarche follows at a mean age of 12.75 years in the US children (Hamill 1979). Growth velocity then slows and growth ceases at about 15 years, with radiological epiphyseal fusion occurring at the radius and ulna at 15.8 and 15.9 years respectively (Hansman 1962).

Boys grow at a median rate of 5 cm/year for a further two years before the onset of their pubertal growth spurt, which commences at a median age of 12 years. There is a wide degree of variation in the rate of pubertal changes, e.g. the range for boys to pass from genital stage 2 to 3 is 0.4 to 2.2 years (Preece et al 1992). Height velocity reaches a peak of 9.5 cm/year at a median age of 13.5 years in the US (Tanner and Davies 1985), and 6 months later in British children (Pan and Radcliffe 1992). Growth ceases at a median age of 18 years, with radiological epiphyseal fusion occurring at the radius and ulna at modal ages of 18.0 and 17.8 years respectively. The mean adult height difference between sexes of 13 cm develops at this time with an extra 10 cm to boys in the two years before the growth spurt plus an extra 3 cm from the more intense height velocity. Thus whilst girls get bigger earlier than boys, boys get bigger than girls eventually.

Parallel to these rapid changes in body size are changes in lean body mass (LBM) and fat mass, with marked differences between the sexes. Between 10 and 17.5 years LBM in girls increases about 40% whilst in boys it doubles (Hamill et al 1979). Fat mass on the other hand increases by 50% in girls but only 20% in boys (Boulton 1981).

What are the concomitant changes in bone mass, size and density? Many authors have related BMC and BMD to age, sex, pubertal status and anthropometric measurements such as height and weight.

2.6.1 Age and gender

Many cross-sectional studies using SPA, DPA and DXA have reported increases with age of BMC in the appendicular and axial skeleton and in the total body in both boys and girls (Tables 2.10 to 2.12). Studies using SPA measurements of the forearm have reported that whilst boys had greater values for BMC and bone size (radial width) from 15 years there were no differences in younger subjects (Christiansen et al 1975a, Geusens et al 1991). Studies using DPA and DXA to measure spinal and total body bone mass have generally shown that there are no sex differences in subjects less than 17 years, but that males of 20 years and over have greater values for BMC and TB BM than females (De Schepper et al 1991, Geusens et al 1991, Bonjour et al 1991, Rico et al 1993c). These observations on BMC of LS reflect the larger size of bones in young adult males compared with females (Bonjour et al 1991, Geusens et al 1991).

When BMD is considered results are slightly different. The majority of studies using SPA, DPA and DXA have reported BMD as an areal measure (aBMD), and have shown that this increases with age. Sex differences in aBMD change with age and site. For example studies on the forearm showed that boys aged 4-16 years had a significantly greater distal radius aBMD than girls (Landin and Nilsson 1981), but that females aged 16-20 had a greater aBMD at the distal radius compared with males (Geusens et al 1991). However males aged 21 to 25 years had a greater aBMD of the distal and proximal radius compared with females (Geusens et al 1991). A similar study on a sample of 500 subjects aged 8 to 17 years by Gunnes (1994) found that there was no sex difference for the proximal forearm (65% cortical) but that aBMD of the ultradistal forearm (65% trabecular) was significantly greater in boys compared with girls.

Studies using DPA and DXA have also shown that the sex differences in aBMD are age and site dependent with girls having a greater aBMD in the earlier adolescent years but boys having a greater aBMD in the late teens and early adulthood (Kroger et al 1992, Faulkner et al 1993, Lu et al 1994), and that the sex differences are less marked in LS than FN (Southard et al 1991, Lu et al 1994).

Overall these studies suggest that the changes in bone density follow, by one to two years, the changes in upper limb muscle strength occurring during puberty and on into young adulthood. aBMD does not fully account for differences in bone size which are of central importance when assessing bone density in children and adolescents. Several studies have attempted to overcome this problem by estimating true bone volume and calculating a volumetric bone density (vBMD). For example, Landin and Nilsson (1981) assumed a cylindrical shape for the radius and reported that in 4 to 16-year-olds there was no sex differences for forearm vBMD although this did increase with age. Lu et al (1995) estimated vBMD of the FS, assuming this also to be a cylinder, on 204, 8 to 27-year-olds and reported no change in vBMD with age in either boys or girls but reported that vBMD was higher in boys than girls across the age range ($p<0.05$). In a study of females aged 9 to 21 years, Katzman et al (1991) found a significant association between age and vBMD (estimated using densitometry-derived area and skeletal length measurements) at LS and mid-radius but not for TB or FN. Kroger et al (1993) estimated vBMD of the LS and FN using hypothesized bone volumes and reported that vBMD of LS increased with age but that vBMD of FN was independent of age.

QCT can be used to measure both cortical and trabecular vBMD of the spine by determining the volume of the vertebral body. Gilsanz et al (1994) showed that in children and adolescents aged 4 to 20 years, the volumes of the vertebral bodies were greater in males than in females and increased with age in both sexes. vBMD also increased with age but there were no sex differences.

These varying results from studies which have used different techniques at different ages and at different sites illustrate the heterogeneity of bone growth with respect to site, gender and age

and according to the measure (BMC, aBMD, vBMD). Whilst much of the increase in BMC and also aBMD can be attributable to increases in bone size under the influence of hormonal changes through puberty, variations in skeletal growth between sites with respect to age and gender will manifest as variations in these two bone mass measurements. If true volumetric density is measured differences in this parameter cannot be attributable to differences in bone size. Measurements of vBMD in the total body, FN and FS indicate that there is no change with age whereas in the lumbar spine vBMD does increase with age. These differences may reflect the nature of the bone that is predominant at these sites namely cortical bone in the TB, FN and FS and trabecular bone in the spine. Within trabecular bone trabecular thickening and even addition of trabeculae may occur without any change to the overall dimensions of the bone. In contrast, cortical bone is composed of compact plates lying in tight apposition to each other, therefore acquisition of additional bone is associated with a simultaneous change in dimensions of the bone. Thus increases in TB, FN and FS bone mass result from increases in bone dimensions and so volumetric density does not change. In the vertebra however bone mass may increase without an increase in bone dimensions and thus there will be an increase in bone density.

This does not explain the increase with age in bone density of the forearm, which is predominantly cortical bone. Katzman et al (1991) proposed that this could be explained by a loss of red marrow from the forearm during maturation which would permit some degree of endosteal bone apposition and thus an increase in bone volume. This is supported by the work of Garn (1970) who showed a progressive diminution of metacarpal medullary diameter in girls aged 10 to 22 years.

A number of cross-sectional studies have reported bone accrual velocity including comparisons between boys and girls (Landin and Nilsson 1981, Bonjour et al 1991, Rubin et al 1993, Gunnes 1994). Such measures can be used to indicate the timing of PBM. Rubin et al (1993) reported that the rate of increase in bone size of the forearm fell before the rate of increase in BMC. Bonjour et al (1991) reported that the point of maximal increase in BMC of LS

occurred later in males (13 to 16 years) compared with females (12 to 14 years), however such a delay was not apparent with respect to pubertal status.

From SPA measurements of aBMD of the forearm Landin and Nilsson (1981) reported there was no significant increase after 15 years in girls but a continuing increase in boys. The upper age limit of 16 years did not permit identification of a fall in the rate of increase. Similarly Rubin et al (1993) reported the increase in radial aBMD was less after 15 years in girls whereas boys still displayed a rapid increase. Gunnes (1994) reported that the timing of increase of aBMD of the forearm was different between the sexes according to the site in the forearm, with increases occurring earlier in boys than in girls at the ultradistal site but earlier in girls at the distal site. Bonjour et al (1991) determined increments of growth in LS, FN and FS aBMD in 207, 9 to 18-year-olds. Increments for FN and FS were greater in males than females but not for LS. They also reported a marked reduction in rate of increase in aBMD in females after 15 years.

Bone growth velocities are more accurately determined from longitudinal studies as these eliminate inter-subject variation (Table 2.14). Theintz et al (1992) measured BMC and aBMD of the LS, FN and FS in 198, 9 to 19-year-olds and again one year later. In females the increase in BMC and aBMD was particularly pronounced from 11 to 14 years but was less after 16 years, and gains were not statistically significant from 17 to 20 years. In males the rate of increase was high from 13 to 17 years and then declined but remained significant from 17 to 20 years in LS and FS but not FN. In a one-year follow-up study of 65, 7 to 20-year-olds Kroger et al (1993) reported that the maximal increases in bone size of LS occurred before menarche in girls but over a longer period in boys. The annual increases in BMC and BW of FN in the age range 7 to 20 years, were significantly greater in males than females but there were no differences in LS. For aBMD the most marked increases in girls occurred between 11 and 13 years (the time of menarche) whilst for boys the greatest increases occurred between 13 and 17 years. This study by Kroger is the only longitudinal study in which vBMD has been estimated. Increases in vBMD followed a similar pattern to those of aBMD with respect to gender but increases were 5-6% per year less for vBMD compared with aBMD.

These observations suggest that the majority of bone mineral has been acquired by 17 years in girls and 20 years in boys, but that the precise timing of maximal increase and peak attainment varies between sites. The evidence for timing of PBM has been discussed.

For a more precise understanding of the changes in bone size, mineral content and density through adolescence longitudinal studies covering the whole period are required. Additionally normalisation of BMD values for size of bones is necessary when changes in BMD are followed because the change in bone size will affect the results. Only one prospective study has been reported in Australia and this was on a small number of subjects (25 boys, 28 girls) and very short . Two measurements were made at a one year interval and only changes in aBMD were reported (Lu et al 1994).

2.6.2 Pubertal status

Bone size, mineral content and density in adolescence are associated with pubertal status. Many of the age-related differences in changes in bone mass between boys and girls may be attributed to the differences in timing of pubertal development. The median age of onset of puberty in girls (defined as stage 2 breast development) is 10.9 years, and in boys (defined as 4ml testes) is 11.5 years (Hamill et al 1979). A number of studies have reported the association between bone mass and pubertal status, although the majority have reported aBMD (Dhuper et al 1990, Glastre et al 1990, Gordon et al 1991, Sentipal et al 1991, Southard et al 1991, Grimston et al 1992, Rubin et al 1993) rather than BMC and BW (Krabbe and Christiansen 1984, Bonjour et al 1991, Rico et al 1993c) and few have used vBMD (Gilsanz et al 1988b, Katzman et al 1991, Mora et al 1994).

These cross-sectional studies have shown that BMC, BW and aBMD are positively associated with pubertal stage as determined by Tanner score. Differences tend to be small between stages 1 and 2, and between stages 4 and 5 but between early and late stages there are large differences in all measurements in both sexes. Whilst for BMC boys had greater values than girls at all stages of development (Bonjour et al 1991), for aBMD there were no sex

differences in the early stages although boys tended to have greater values than girls in the later stages (4 and 5) (Bonjour et al 1991, Rico et al 1993c).

Katzman et al (1991) in their study of 45 females reported that Tanner score was not correlated with vBMD at LS, FN or TB, but was weakly associated with vBMD at mid-radius. Gilsanz et al (1988b) who used QCT to determine vBMD, reported a highly significant effect of puberty on spinal trabecular and cortical vBMD. Mora et al (1994) also measured spinal vBMD by QCT in 96 girls aged 4 to 20 years, and reported that cortical vBMD increased with each stage of puberty whereas trabecular vBMD was only greater at the later stages (4 and 5), thus demonstrating substantial differences in timing of mineral accretion between cortical and trabecular bone with respect to pubertal development.

Rubin et al (1993) proposed that there were two parallel but distinct aspects of skeletal growth during puberty, namely, accelerated linear growth and accelerated bone accretion both mediated by hormones and growth factors. They suggested that the hormonal effects of puberty were stronger in determining axial aBMD of LS (predominantly trabecular bone) in comparison with peripheral aBMD of the radius (predominantly cortical bone). The onset of the age-related accelerated phases of axial aBMD accretion corresponded to the time of onset of the pubertal growth spurt in both sexes (early puberty in girls and mid-puberty in boys). Radial aBMD displayed steady growth related increases throughout most of childhood and adolescence.

A study by Gordon et al (1991) in which BMC and aBMD of LS were measured in 236 subjects aged 3-30 years, found that aBMD was marginally greater in females compared with males but that BMC was greater in males due to the larger cross-sectional area of the vertebra. The only increases in BMC and aBMD after age 10 in females were associated with puberty, whereas in males these measures increased steadily throughout the age range 3 to 30 years, and the increases associated with puberty were smaller in males compared with females. They proposed that growth during puberty contributes less to peak bone mass in males (21% BMC, 11% aBMD) than in females in whom 55% BMC and 39% aBMD accumulates at the time of

puberty, and that bone mass does not increase after the early teenage years. Although changes in pubertal stage are closely associated with age, they are highly variable between individuals in both the timing of onset and tempo. It is therefore difficult to differentiate between their effects with respect to bone growth.

Age at menarche in girls may also be used as a measure of sexual maturity. A study in premenopausal women aged 19 to 40 years found that those women in the lowest quartile for aBMD of LS had had a significantly later age at menarche compared with those in the upper quartiles (Armamento-Villareal 1992). Lu et al (1994) reported that in 50 post-menarchic subjects there was no association between the age of onset of menarche and aBMD of TB, FN and LS, although the aBMD of TB was related to the number of years post-menarche for up to 2 years only. Turner et al (1992) reported a trend to a lower aBMD of LS with menarche after 15 years in a study of 15 to 17 year-olds. However those with late menarche (after 15 years) would have been within 2 years of menarche and so within the period of rapid increase. Several other studies have reported that increases in aBMD are greatest in the first 2 years post-menarche and that increments in the following years are much less (Gunnes 1994, Theintz et al 1992).

The effect of pubertal status on bone mass has not previously been reported in Australian children, nor has the rate and extent of change in bone growth been previously related to the whole range of stages of puberty in the one sample.

2.6.3 Measures of body size

Bone mass measurements have been related to indices of body size in a number of studies. Strong associations between BMC and bone size at many sites and height and weight have been reported (Chan 1991, Landin and Nilsson 1981, Katzman et al 1991, Rubin et al 1993). Several studies have estimated lean tissue mass using DXA. Faulkner et al (1993) from a study of 234 children aged 8 to 16 years, reported that bone free lean tissue was the most important predictor of TB BMC in both boys and girls. Similarly Ogle et al (1995) reported a strong

relationship for both sexes between lean tissue mass and TB BMC in male and female subjects aged 4 to 26 years. These findings suggest that peak BMC is determined by the amount of lean tissue which in turn is a function of body size. Such results are not unexpected as BMC is a function of bone size which in turn reflects body size.

Significant associations between aBMD, which partially corrects for bone size, and measures of body size have also been reported but such associations are generally not as strong as those for BMC and bone size (Katzman et al 1991, Lloyd et al 1992). One study in which vBMD of the spine was determined by QCT in 96 females aged 4 to 20 years, reported a significant association of height, weight, body mass index and muscle volume with cortical but not trabecular vBMD (Mora et al 1994). The authors proposed that this discrepancy between the different bone compartments was consistent with the view that weight-bearing stresses and /or mechanical factors are important determinants of cortical bone density in the axial skeleton, but are less important regulators of trabecular bone density, during growth. In contrast Lu et al (1995) who estimated vBMD of the FN and FS (predominantly cortical bone) in boys and girls aged 8 to 27 years reported these to be independent of height. These differences may reflect the differences between the weight-bearing axial skeleton and the appendicular skeleton.

Changes in height and weight have been associated with changes in bone measurements. Goslings et al (1995) reported a significant association between height and weight velocity, and bone mineral accrual and bone width velocity of the distal radius in 6 to 12-year-olds. Similarly Kroger et al (1993) reported annual height gain to be significantly associated with increases in BMC, BW and aBMD at the LS and FN but there were no such associations for vBMD. Theintz et al (1992) in their one year longitudinal study found that changes in height and changes in BMC of the LS were related to pubertal stage, but that a much greater increase in bone mass for the same increase in height occurred at stages 3 and 4 in both males and females. These associations between height velocity and bone mass velocity reflect the relationship between BMC and bone size. When bone size is controlled for as in the measurement of vBMD there is no such relationship.

There are few reports in children and adolescents regarding the association between bone mass and body fatness. Miller et al (1991) reported a wide range of anthropometric measurements including a number of skin-fold measures in 140 boys and girls aged 5 to 14. Whilst at all skeletal sites skinfolds were negatively correlated with aBMD, fatness measures were seldom significant predictors of skeletal densities. A study by Rico et al (1994) in which TB aBMD was determined in 50 post-pubertal females aged 14 to 18 years, reported that this measure was correlated with lean body mass but not with percentage body fat. Similar findings have been reported in young women (Aloia et al 1995), in contrast to the finding in older adults and postmenopausal women of a protective effect of fatness with respect to bone mineral density (Mazess et al 1990, Mazess and Barden 1990, Slemenda et al 1990, Reid et al 1992).

2.6.5 Skeletal maturity

Bone age is the most precise indicator of skeletal maturity. Skeletal maturity is defined radiologically as the point at which epiphyseal-diaphyseal fusion is complete. In boys this is defined as a bone age of 19 years and occurs at a modal age of 18.0 and 17.8 years for the radius and ulna respectively. In girls skeletal maturity is defined as a bone age of 18 years and the corresponding modal ages are 15.8 and 15.9 years for the radius and ulna respectively (Greulich and Pyle 1959, Hansman 1962). Longitudinal bone growth does not occur beyond this point. A number of studies have reported bone age to be highly correlated with BMC, BW and aBMD (Glastre et al 1990, Mazess and Cameron 1971) and vBMD (Trouerbach et al 1991). It is of particular relevance in terms of the possible beneficial effects of lifestyle intervention in youth as to whether bone mineral accretion is complete when skeletal maturity is reached, or whether it can continue into early adulthood. None of the above studies have addressed this point.

2.6.6 Environmental and genetic influences

The roles of nutrient intake and exercise in determining PBM have been discussed. At the time of the pubertal growth spurt there are concurrent changes in appetite. These changes are

complicated by social pressure on girls to be thin resulting in a high incidence of dieting with potential to limit particular nutrients. Because of these social pressures many adolescents do not meet the recommended requirements for calcium intake (DCSH 1989, Magarey and Boulton 1994b). As pressures of school work increase exercise levels also typically decrease, especially in girls (Boulton 1981). How do these lifestyle changes of restricted nutrient intake and lower exercise levels affect bone growth and mineral density and the attainment of PBM?

The role of genetic effects on determining PBM has been discussed. Genetic effects on bone mass may be mediated through body weight and height, hormonal levels, metabolic differences in calcium metabolism and differences in laying down of bone. Whilst several studies have estimated the contribution of genetic factors to the variance of bone mass by parent-child comparisons these have been on young adults (Tylavsky et al 1989, Henderson et al 1995), or if including adolescents have studied only daughters with mothers and fathers (Matkovic et al 1990) or sons and daughters with only mothers (McKay et al 1994). No previous study has included adolescent sons and daughters and both parents.

2.7 CONCLUSION

The human skeleton is formed and modelled during infancy, childhood and adolescence through a range of genetically determined effects and the influence of mechanical loading. As the skeleton grows in length the bones expand in diameter. Following cessation of longitudinal bone growth consolidation of bone continues into young adulthood. Although the majority (over 90%) of bone mass is attained by about 17 years in girls and 17 to 20 years in boys depending on the site of measurement, PBM is not reached at some sites (eg forearm) until the end of the third decade or possibly later. The relation of attainment of PBM to skeletal maturity is not known. Since cessation of longitudinal growth occurs at different times in different bones, measurement of forearm bone mass allows comparison of radiologically determined skeletal maturity at that site with bone mass and bone mass changes at that site.

The prepubertal and pubertal years are a time of rapid bone growth in both boys and girls. The assessment of whether real differences in bone mineral density, and hence bone strength, occur at different stages of maturity, and whether these differences are due to different levels of activity and nutrient intake, is to some extent confounded by the difficulty of accounting for the change in bone volume when BMD is determined as an areal measure rather than a volumetric measure. Use of SPA as modified by Nordin et al (1986), enables estimation of a volumetric bone density that takes account of the changing bone dimensions in this period of rapid growth. During this time bone mineral content, bone size, and areal bone mineral density are known to be associated with age, gender, pubertal stage and variables of body size. The role of each of these variables depends on the bone measurement under consideration and the site of the measurement (eg axial or appendicular or total body).

Almost all our understanding of these changes comes from cross-sectional or short-term longitudinal studies. In addition there are few Australian data on bone mineral content, bone width and bone mineral density in normal children, nor changes in these measures as children progress through puberty. The Adelaide Nutrition Study cohort provided a unique opportunity to redress this.

Given the importance of calcium in bone mineralisation and the role of exercise in maintaining bone health, it is pertinent to measure these variables and determine if variations in calcium intake and level of exercise as observed in normal free-living adolescents are significant predictors of bone mass. Whilst several studies have reported the relationship between parental and children's bone mass few have included fathers and mothers of boys and girls. No Australian studies have documented this relationship nor determined if parental bone mass is a significant predictor of bone mass and rate of change in bone mass in the adolescent years.

2.8 AIMS and HYPOTHESES

The aim of this longitudinal study was to document the changes in forearm bone mineral content, bone width and bone mineral density in a cohort of Australian born, Caucasian boys and girls between the ages of 11 and 17 years and to relate these changes to dietary intake and physical activity.

These data were used to test the hypotheses:

1. that peak forearm bone mineral content, width and density are reached at skeletal maturity as defined by the cessation of longitudinal growth,

2. that the significant predictors of forearm bone mineral content, width and density, and rate of change in these variables through the adolescent period 11 to 17 years are:

- age
- gender
- sexual maturity
- body size
- nutrient intake
- physical activity
- parental bone mass.

CHAPTER 3

METHODS

3.1 SUBJECTS AND METHODS

3.1.1 The subjects

The subjects were selected from the Adelaide Nutrition Study. This study recorded the growth and nutrient intake of a group of Adelaide children from birth in 1975-76, through infancy and childhood (Boulton 1981, Magarey and Boulton 1987). In 1986, when subjects were aged 11 years, 105 of the 130 were selected on the basis of their previous co-operation in completing questionnaires and attending appointments and invited to participate in the present bone density study. One hundred, 56 boys and 44 girls agreed to take part. An additional 8 girls were selected from an age-matched cross-sectional sample of 703 children who had taken part in a family heart disease risk factor precursor study when aged 8 years (Boulton et al 1995a). At the time of enrolment participants agreed to have bone density measurements taken at age 11, 13 and 15 years.

At 11 years there were 108 subjects, 56 boys and 52 girls. At age 13, 54 boys and 49 girls participated. One girl had moved interstate, two girls and one boy declined to participate and one boy failed to keep appointments. At 15 years, all boys who participated at 13 years and the one boy who broke appointments at 13 years took part, and all but one of the girls from age 13. This girl had moved address and could not be traced.

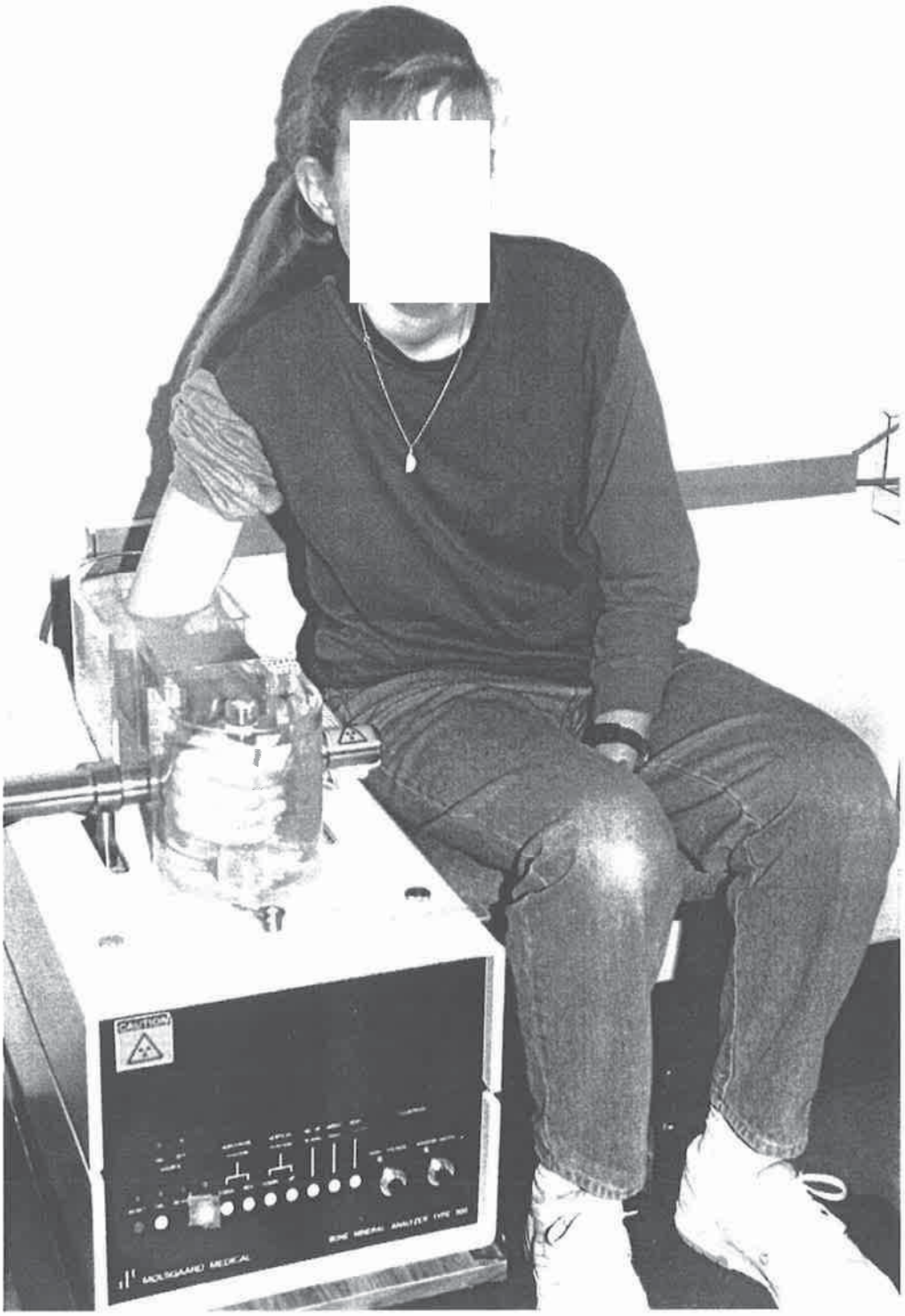
At 17 years all those who participated at 15 years were invited to have another bone density measurement. 51 boys and 42 girls agreed to take part. One boy and one girl who had participated in the Adelaide Nutrition study to age 15 years also had their bone density measured at 17 years. Of those who did not participate, one girl was pregnant, three girls and two boys declined despite the wishes of the parents of the boys, one boy and two girls had family circumstances which made it difficult to attend and keep the diet record, and one boy and one girl failed to keep repeated appointments.

Six months prior to the 17 year measurement, parents of all those subjects seen at 15 years were invited to have their bone density measured. Of the 105 families, 78 fathers and 99 mothers took part. The lower number of fathers was due to deceased (3), living interstate (2), no longer living in the household and no contact with family, or no response (9), unable to attend due to work commitments (2), and declined (5). A number of fathers participated although they were no longer living with the subject. Parents of two subjects participated although their sons did not attend at 17 years; in four families personal circumstances prevented the parents' participation; and parents of two subjects both declined. Table 3.1 shows the number of subjects at each age and number of parents, by sex.

3.1.2 Bone measurements

All bone measurements on the subjects and their parents were performed in the Department of Nuclear Medicine, Royal Adelaide Hospital. Forearm bone density was measured with a Molsgaard Bone Mineral Analyzer using I^{125} as the gamma-ray source. The right arm was measured unless a fracture had been sustained in this arm. One subject had fractured both arms prior to the first measurement, and in this case the arm in which the fracture was less recent was measured. With the forearm in a waterbath (Figure 3.1), the distal radius and ulna were scanned six times moving proximally at 4 mm intervals from the first point where the interosseous space exceeded 8 mm. An attenuation tracing (Figure 3.2) representing the scan of the two bones was produced by an HP85 desktop computer which converted forearm mineral content from arbitrary units into true mineral units after calibration against an ashed radius and ulna. This produced a result for forearm bone mineral content (BMC) in g/cm (Nordin et al 1986). The negative absorbance caused by fat tissue was accounted for by adding the integral of this negative deflection above the baseline, to the measured BMC to give a fat-corrected BMC (Need et al 1989). Bone width (BW) was also calculated by the computer using an edge detector programme. Dividing BMC by the BW produced a 'surface density' measurement of g/cm^2 here called areal bone mineral density (aBMD). Dividing BMC by the estimated cross-sectional area of the bones produced a volumetric forearm mineral density (vBMD) in g/cm^3. Bone area was calculated for the ulna by assuming the bone was tubular in

Figure 3.1
The densitometer measuring the forearm bone density of a 15-year-old subject. Note the arm immersed in a water bath and the hand in a fixed position


CAUTION
MOLDGAARD MEDICAL

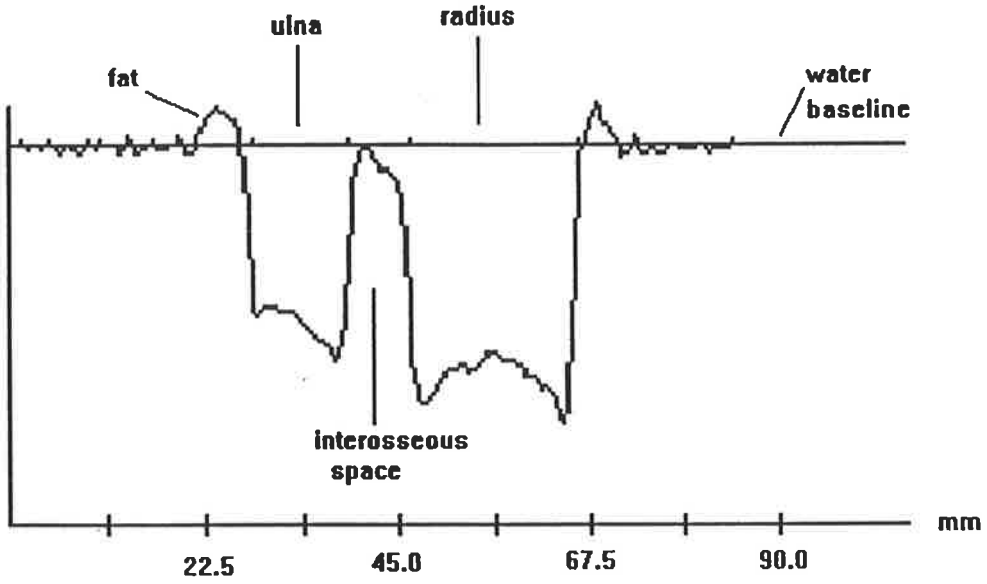


Figure 3.2
The tracing produced by the densitometer showing attenuation of the gamma rays by the two bones and their transmission through the interosseous space, through water and through fat.

cross-section, and for the radius using the data of Horsman and Leach (1974), which at the scan site yields a radial area of [(15.2 x radial width) - 95] (Nordin and Polley 1987).

The instrument was calibrated daily using a bone phantom made of aluminium. The error on BMC was 1.0% (Nordin et al 1986), and on the fat corrected BMC 1.7% (Wishart et al 1991). The error on BW was 1.9%, and the error on the area measurement 3.4% (Nordin et al 1986).

At the selected point of the forearm the composition of the bone is approximately 80% cortical and 20% trabecular (Schlenker and Von Seggen 1976).

vBMD in post-menopausal mothers was corrected for bone loss using the following formula, which proportions bone loss in the forearm to age and years since menopause (Nordin et al 1990).

BMD_{corr} = BMD + 4.1 x (age over 55 in years) + (15.8 x $\log_e$ (years since menopause)).

For comparison of the subjects' values with adult values, fat corrected BMC and vBMD derived in the same laboratory were used. These were based on 100 premenopausal women aged 18 to 58 (mean 38.9 years) (Wishart et al 1991) and 90 men aged 22 to 50 years (Wishart et al 1995). The values were men: BMC 1.707 g/cm, vBMD 516 g/cm^3, and women: BMC 1.175 g/cm, vBMD 483 g/cm^3.

3.1.3 Anthropometric measurements.

Each subject, in indoor clothing and without shoes, was weighed to the nearest 100g using a beam balance and measured for height to the nearest mm using a Holtain stadiometer (Holtain Ltd. Crymych, Dyfed, UK). Skinfold thickness was determined as the sum of skinfold thicknesses at the biceps, triceps, subscapular and suprailiac, measured to the nearest 0.1 mm using Holtain skinfold callipers (Holtain Ltd. Crymych, Dyfed, UK). Two measurements were taken at each site and averaged. All anthropometric measurements at every age were made by the author.

3.1.4 Bone age

Bone age was determined at the time of the 17 year bone measurement in the Department of Organ Imaging Women's and Children's Hospital according to the method of Greulich and Pyle (1959). The radiographs were estimated by an independent radiologist.

3.1.5 Pubertal status and sex hormones

Pubertal status was self-assessed by each subject being shown standard photographs and explanations of Tanner stages (Tanner 1962, Duke et al 1980). This procedure was chosen as being the least embarrassing for the subjects and therefore ensuring maximum compliance. Assessment by a physician was not performed. At each visit girls were asked if they had commenced menstruation since the previous visit and if so their age at the time of menarche.

At 11, 13 and 15 years serum testosterone was measured in boys and serum oestradiol levels in girls. Results are not available for all subjects as a result of insufficient and loss of blood samples. Blood was primarily collected for lipid analyses. All hormone levels were measured in the Department of Endocrinology at the Queen Elizabeth Hospital, Adelaide. Testosterone was determined using the Farmos kit (Farmos Diagnostica, Oulunsalo, Finland) liquid phase radioimmunoassay (RIA) using double antibody separation and I^{125} as tracer. At ages 11 and 13 years oestradiol was determined using the Farmos kit (Farmos Diagnostica, Oulunsalo, Finland). This is a second antibody coated tube RIA using I^{125} as tracer. At 15 years oestradiol was determined using the Serono kit (Serono Diagnostics Ltd, Woking, Surrey, UK). This is a liquid phase RIA using I^{125} as tracer and magnetic separation.

The time of blood collection with respect to the menstrual cycle was not recorded.

3.1.6 Dietary intake

Nutrient intake was estimated from a 4-day weighed food record. Detailed instructions prepared by the author and record forms were sent to each family shortly before the child's

birthday at 11, 13 and 15 years and prior to the final bone measurement when subjects were 17 years. Specific dates were suggested for diet recording starting on a Sunday. Food items were recorded as weight (g) or volume (metric cup (ml), level teaspoon or tablespoon). For commercial items such as sliced bread, packaged biscuits, meat pie, details of the item and the number of units consumed were recorded. Weights were calculated by the author from the number of slices per loaf and total weight of the loaf etc. For dishes cooked at home weights of individual ingredients, final weight (or number of serves e.g. biscuit, scones) and weight or proportion eaten by the subject were recorded. Weight of uneaten food at the end of a meal was also recorded. For the occasional meal purchased at restaurants, estimates of ingredients and weights were made. All records were discussed with the author on attendance for measurements, and necessary details added. Subjects who had been part of the Adelaide Nutrition Study, had completed weighed food records at age 4 years (3 days), and 6 and 8 years (4 days).

Not all subjects completed the diet record every time. Several subjects (particularly as they became older) agreed to participate in the bone measurements on the understanding that they need not complete the diet record. A number of other subjects who failed to keep the record prior to the appointment also failed to return it at a later date. One girl at 17 years said it was impossible to get a representation of her diet by recording four days. The records of one boy at 11, 13 and 15 years and a second boy at 13 and 15 years were rejected as it was thought these were not a true record of intake for the four days. Table 3.1 shows the number of records analysed at each age.

The diet records were analysed using the Diaryan nutritional data bank (CSIRONET 1980) which is based on McCance and Widdowson's 'The Composition of Foods' (Paul and Southgate 1978). The data bank includes a number of foods with Australian analyses and selection of the appropriate food code was assisted by a field of manufacturers' data collated in Adelaide. It was often necessary to use more than one code for a particular food. Prior to the analysis of the records at 13 years, 43 new foods were added to the data base. Some of these were foods for which there had been no previous analysis, others replaced British foods which

are significantly different in some nutrients due to different practices of nutrient fortification, for example, iron in breakfast cereals and calcium in flour which affects the analysis of bread and all baked products using flour. Four new foods were added to the data base prior to analysis of the 15 year diet records.

In view of the importance of calcium intake to this study the 11 year diets were recoded and reanalysed using the amended data base which used Australian data for bread and flour thus reducing considerably the error associated with calcium estimation. This correction resulted in a mean decrease in daily calcium intake for the sample of 61 mg (7%). The percentage decrease was greater in those subjects with lower calcium intakes. The major foods for which calcium content would be overestimated were then cracker biscuits and commercial sweet biscuits and cakes which contribute less than 5% to calcium intake (Magarey and Boulton 1995a).

Energy and nutrient intakes were calculated as the mean daily intake.

3.1.7 Physical activity

A physical activity score was determined for each subject at each age. At ages 11,13 and 15 years each subject completed a questionnaire to determine usual daily activity. Questions related to means of travel to and from school, usual level of activity at mid-morning break and lunch time, after-school activities, and participation in regular sporting activities including competitive sport. Subjects also completed a 7-day record of physical activities and outside day-to-day living activities. At 17 years due to the wide-ranging life-styles of subjects as a result of many of them no longer attending school, subjects completed a detailed 7-day diary of activity including time spent sleeping, in sedentary and ambulatory activities, and in sporting activities.

From 11 to 15 years, a weekly activity score was calculated by assigning the following hours per week to each activity

travel to and from school by walking or cycling	2.5
mid-morning break : active 5 x 0.25	1.25
lunch : active 5 x 0.75	3.75
: moderately active 5 x 0.5	2.5
after school : active 3 or more times per week	2.5
: moderately active/active 1-2 times per week	1.25
each sport played per half year	0.5
11 years only	
health hustle daily	1
regular PE class	1

as well as at each age the number of hours spent in other activities of 30 minutes minimum duration, according to the activity diary.

At 17 years the number of hours per week spent sleeping, sedentary, in domestic duties, walking (including work requiring standing), and moderately and strenuously active was determined from the 7-day diary. The weekly activity score was calculated as the sum of

(i) the number of hours moderately and strenuously active

(ii) half the number of hours ambulatory

(iii) quarter of the number of hours in domestic duties greater than 2 hours per day

The weekly activity score was designed to stratify subjects into activity level and not to estimate energy expenditure. In assigning hours to activities consideration was given to the benefit of weight-bearing exercise and use of muscles, in bone turnover and growth.

3.1.8 Data analysis

All data was analysed using the Statistical Package for the Social Sciences personal computer version, SPSS PC+ (Norusis 1988). Descriptive statistics (mean, standard deviation(sd) and standard error (sem)) were obtained for all bone and continuous variables. All variables were tested for normality.

For all statistical tests significance was defined as $p<0.05$ (Rothman 1986).

Standard t-tests and analysis of variance (ANOVA) were used for comparison between groups. The relation between continuous variables was evaluated by means of the Pearson correlation coefficient and univariate regression analysis.

The goodness of fit of the regression line of each bone variable on age was determined from the F statistic of the ratio of the regression mean square to the residual mean square.

Two factor repeated measures MANOVA was used to analyse the effect of gender and age on each of the bone variables and on the change in each bone variable. The assumption of homogeneity of variance was checked by the Mauchley Sphericity test to avoid the associated bias of the F test.

Two factor ANOVA was used to determine the effect of gender and pubertal status (PS) on each bone variable. For the subsequent comparison of bone values at the different pubertal stages, the Tukey multiple range test was applied. Three factor ANOVA was used to determine the effects of age, gender, and pubertal status on each bone variables.

Estimates of heritability between subjects and parents were calculated as the ratio of covariance of child and parent to the variance of parents ie the regression coefficient (beta) of child on parent value. This method estimates the additive heritability due to parental influence and is a decimal fraction between 0 and 1.0 (Tylavsky et al 1989).

The ability of variables describing biological factors (age, pubertal status, anthropometric measurements), environmental factors (nutrient intake, physical activity), and genetic factors (parental bone values), to describe the variance in each of the bone variables and the change in each bone variable, was examined by multiple linear regression analysis. All the predictor variables were initially included in the regression model. One by one the least non-significant predictor variable (largest p value) was deleted until only significant predictor variables remained. Subsequently each non-significant variable was added to the final equation. Sex and each pubic hair stage were coded as dummy variables, sex: 0 = male, 1 = female; PHS1: 1 = phs of 1, 0 = phs of 2 to 5; PHS2: 1 = phs of 2, 0 = phs of 1 and 3 to 5 etc.

3.1.9 Power determination

The power of this study to detect a statistical difference between two means (eg boys and girls, time 1 and time 2) based on a medium effect size of 0.5, a one-tailed significance of 0.05 and a sample size of 50 was 80% (Welkowitz et al 1982). The power of this study to detect a significant correlation of 0.30 (eg between calcium intake and BMC), equivalent to a medium effect size, assuming a sample size of 50 and a one-tailed significance level of 0.05 was 68%. For the smaller sample size of 43 (number of girls at 17 years) the power was 62%.

A medium effect size of 0.5 is equivalent to a difference between two menas of half the sample standard deviation. In menopausal women a decrease in forearm BMD from 1 to 2 sd units below the mean, increases the fitted odds ratio for adjusted relative fracture risk from 2 to 2.8, and a further decrease to 3 sd units below the mean increases the fitted odds ratio to 4. Since fracture risk as a function of bone density is a continuum, any loss is a disadvantage and conversely any gain in youth and young adulthood is advantageous.

3.1.10 Ethics approval

Ethics approval for measurements at 11, 13 and 15 years was granted by the Ethics Committee of the Adelaide Children's Hospital (now The Women's and Children's Hospital). For the measurements at 17 years approval was given by the Ethics Committees of The Women's and Children's Hospital, The Royal Adelaide Hospital, and the University of Adelaide.

3.1.11 Consent

Signed, informed consent was obtained from both the subjects and a parent for measurements at 11, 13 and 15 years. At 17 years signed informed consent was given by the subjects and similarly by the parents for their own measurements.

Table 3.1

Number of each measurement at each age and number of parents for boys and girls.

	11 years	13 years	15 years	17 years
Boys				
Bone measures	56	54	55	52
Height	56	54	55	52
Weight	56	54	55	52
Skinfold thickness	56	54	54	51
Pubertal status	56	54	55	52
Testosterone	35	42	53	
Bone age				48
Dietary intake	54	52	52	44
Physical activity	57	55	54	43
Fathers				43
Mothers				54
Girls				
Bone measures	52	49	48	43
Height	52	49	48	43
Weight	52	49	48	43
Skinfold thickness	52	49	47	41
Oestradiol	33	38	46	
Pubertal status	52	48	48	43
Bone age				40
Dietary intake	52	49	47	38
Physical activity	52	48	48	37
Fathers				35
Mothers				45

3.2 DISCUSSION OF METHODOLOGY

3.2.1 Bone measurements

SPA was chosen as it was the only method available in Adelaide at the time of the initial measurement in 1987. As this was a longitudinal study it was important not to change methodology part way through when DXA became available.

Longitudinal measurements of bone density will be confounded by changes in bone size. Estimation of bone volume of the forearm enables determination of a volumetric bone mineral density (vBMD) and thus eliminates differences in bone size. This is not possible in the alternative methods of DPA and DXA except for the long bones such as the FS.

There is the potential problem in a longitudinal study during growth of the subjects, that the point of bone estimation is not exactly the same at each measurement due to longitudinal growth of the bones.

3.2.2 Dietary Intake.

There is no single method of estimating dietary intake that can be a precise measurement of overall intake over a long period. When using the weighed food record, the greater the number of days for which the record is kept the greater the precision in estimating overall intake. Selection of a 4-day weighed food record was a compromise between compliance and precision.

There are a number of areas for potential error common to all dietary intake methods.

The data base

All results from dietary surveys are dependent on the quality of the food tables, the number of foods in the data base, the accuracy of the data and the nutrients analysed. The Diaryan program, based on McCance and Widdowson Composition of Foods, is of British origin. The advantage of this programme when dietary analysis was initiated in 1979 when these subjects

were aged 4, was the wide range of items it included. The programme also had the facility to add new analyses and this enabled addition of new foods and foods with obvious differences in nutrient analysis. The disadvantage was the potential difference between British and Australian foods. With respect to this study the most important of these was the fortification in Britain of flour with calcium, affecting all bread and baked products containing flour: biscuits, cakes, pies etc. This problem was minimised by replacing analyses for bread, flour and frequently consumed baked products such as pies and sausage rolls with Australian analyses with the appropriate calcium content. These changes were not made until after analysis of the 11 year data, however these records were recoded and analysed using the revised analyses for bread etc. This change resulted in a decrease in estimated calcium content of 61 mg (7%) per day for the total sample. Dietary intakes from 4 to 8 years were not recoded and reanalysed to correct for calcium in flour.

Another potential source of error when estimating calcium content was the small number of fresh milk items in the tables. Over the 6 year period, 11 to 17 years, there was an increase in the variety of fresh milks available in Adelaide. These milks varied in energy, fat and calcium content depending on whether they were fat reduced and/or calcium fortified. Addition of analyses for these varied milks to the data base ensured accurate estimate of calcium from this source. For the large number of flavoured milks available, manufacturers' information printed on the packaging was used to select the appropriate food code to ensure correct fat and calcium estimation. At each record period new foods, unavailable at the time of the previous record, were included. Data obtained from manufacturers enabled these foods to be added to the data base. Manufacturers' data also enabled selection of the most appropriate food code (often a combination of several codes) for such items as icecreams, sweet and cracker biscuits, and confectionery.

For cooked dishes it is impossible to select a food code that accurately reflects the nutrient content. These subjects consumed many composite dishes e.g. desserts, casseroles, soups, cooked from raw ingredients at home. The weighed food record enables itemisation of the

ingredients of such dishes, and with information of the total weight of dish and weight consumed by the subject, the proportion of each item can be determined.

When subjects consumed foods purchased away from home e.g. in a restaurant, ingredients and weights had to be estimated. However the number of food items recorded in this way was very small over the four day period with less than 1% of meals eaten at a restaurant (Magarey and Boulton 1995b).

Data handling

Coding errors can arise through mistakes or difficulties in interpretation. Mistakes were minimised because all records were coded by the author. The analysis program includes two forms of checking for coding errors, these were a printout of all food items for which weights were greater than 300g, and listing of use of unusual foods. Each of these was checked against the food record.

Accuracy of recording

The precision of spring balances used in dietary surveys is rarely better than +/- 5 g. Balances used by subjects in this study were household scales and varied between households. Errors may also occur due to the skill of the recorder. Individual food items such as slices of bread and commercial biscuits were recorded as the item ie one slice of x brand bread. Weights of these were determined from the loaf weight and number of slices, similarly for biscuits. Items such as spreads, sugar, breakfast cereals and small quantities of vegetables were recorded as level teaspoon or tablespoon measures or cup measures. Information determined in Adelaide, and from manufacturers for weights of these items, was used to convert volume to weight. It was often possible to check accuracy of weighing by comparing the recorded weight of a commercially prepared item with the known manufacturers weight. Meals consumed in restaurants could not be accurately weighed however these accounted for less than 1% of meals. Foods purchased in fast food chains were described and published weights used. Subjects were requested to carry the record with them at all times and record food or drink at the time of consumption.

Change in diet and response bias

When subjects are asked to keep a record of everything they eat and drink there is always a risk that they will alter their eating pattern to make the task easier, or alter it to appear to be having a healthy diet or because they are made aware of how much they are eating. These alterations may be conscious but can also be unconscious.

The wide range of foods, the many recipes included in the records, and the inclusion of foods for which subjects felt they had to apologise at interview, suggests that these alterations were minimal. The time required to record home-cooked dishes may have caused some changes to the family menu but overall there was no indication that major changes were made. Subjects had no reason to display that their diet was 'good', no judgement was made on their intake and they were not expected to be adhering to a prescribed diet.

Given these potential sources of error the weighed food record enables an accurate estimation of intake over the period of recording.

3.2.3 Physical activity

The subjects in this study were normal children participating in a range of organised sports and leisure activities. The relative mechanical load produced by various activities is not known. The physical activity score was designed to quantify the time spent in load-bearing activities and therefore muscle-building, and thus rank individuals for the mechanical load placed on the skeleton.

At 17 years when many of the subjects were no longer at school and the activities engaged in were even more wide-ranging, the 7-day activity diary was used to estimate time spent in each level of activity: sedentary, semi-ambulatory, ambulatory, and moderately to strenuously active. Scoring assumed that the mechanical load in active sports (football, netball, running) was greater than that for low level activities such as walking, which in turn was greater than that for standing with occasional walking (shop assistant, domestic activities).

As there is a degree of uncertainty as to whether strain applied at a particular site can also have an effect on bone metabolism at a distant site, this physical score is a crude estimate of the stimulus being applied to the skeleton or to a particular bone.

CHAPTER 4

RESULTS: BONE STATUS AND BIOLOGICAL VARIABLES

4.1 Age and gender

4.2 Pubertal status, age at menarche, sex hormones

4.3 Anthropometric measurements

4.4 Bone age

4.1 AGE AND GENDER

4.1.1 Absolute bone status

The mean, standard deviation (sd) and range at each age for boys and girls for the bone variables bone mineral content (BMC) as g/cm, bone width (BW) measured in centimetres, and bone mineral density (BMD) as an areal measure (aBMD) in g/cm^2 and as a volumetric measure (vBMD) in g/cm^3, are presented in Table 4.1.1. Figures 4.1.1 to 4.1.4 present the data by age and sex as mean and standard error of the mean (SEM) in those subjects for whom there were measurements at every age (50 boys, 42 girls).

Table 4.1.1

Mean, standard deviation (sd) and range of the bone variables at each age in boys and girls

Age years	11	13	15	17
Boys				
n	56	55	55	52
BMC g/cm				
mean : sd	0.77 : 0.11	0.89 : 0.16	1.19 : 0.25	1.47 : 0.25
range	0.56 - 1.04	0.60 - 1.33	0.76 - 1.77	1.01 - 2.12
BW cm				
mean : sd	2.48 : 0.25	2.83 : 0.31	3.09 : 0.32	3.26 : 0.29
range	1.97 - 2.91	2.26 - 3.50	2.53 - 3.93	2.57 - 3.85
aBMD g/cm^2				
mean : sd	0.31 : 0.02	0.31 : 0.03	0.38 : 0.06	0.45 : 0.05
range	0.26 - 0.37	0.26 - 0.39	0.27 - 0.49	0.33 - 0.55
vBMD g/cm^3				
mean : sd	0.37 : 0.04	0.34 : 0.03	0.39 : 0.05	0.44 : 0.05
range	0.30 - 0.47	0.28 - 0.42	0.39 - 0.49	0.33 - 0.59
Girls				
n	52	48	48	43
BMC g/cm				
mean : sd	0.68 : 0.10	0.84 : 0.15	1.05 : 0.13	1.15 : 0.13
range	0.49 - 0.92	0.56 - 1.12	0.76 - 1.32	0.91 - 1.40
BW cm				
mean : sd	2.41 : 0.22	2.69 : 0.22	2.73 : 0.18	2.77 : 0.20
range	1.86 - 2.77	2.24 - 3.09	2.36 - 3.11	2.40 - 3.09
aBMD g/cm^2				
mean : sd	0.28 : 0.03	0.31 : 0.05	0.39 : 0.04	0.41 : 0.03
range	0.21 - 0.35	0.23 - 0.42	0.29 - 0.45	0.33 - 0.47
vBMD g/cm^3				
mean : sd	0.34 : 0.05	0.35 : 0.05	0.42 : 0.05	0.46 : 0.04
range	0.26 - 0.50	0.24 - 0.47	0.32 - 0.52	0.35 - 0.57

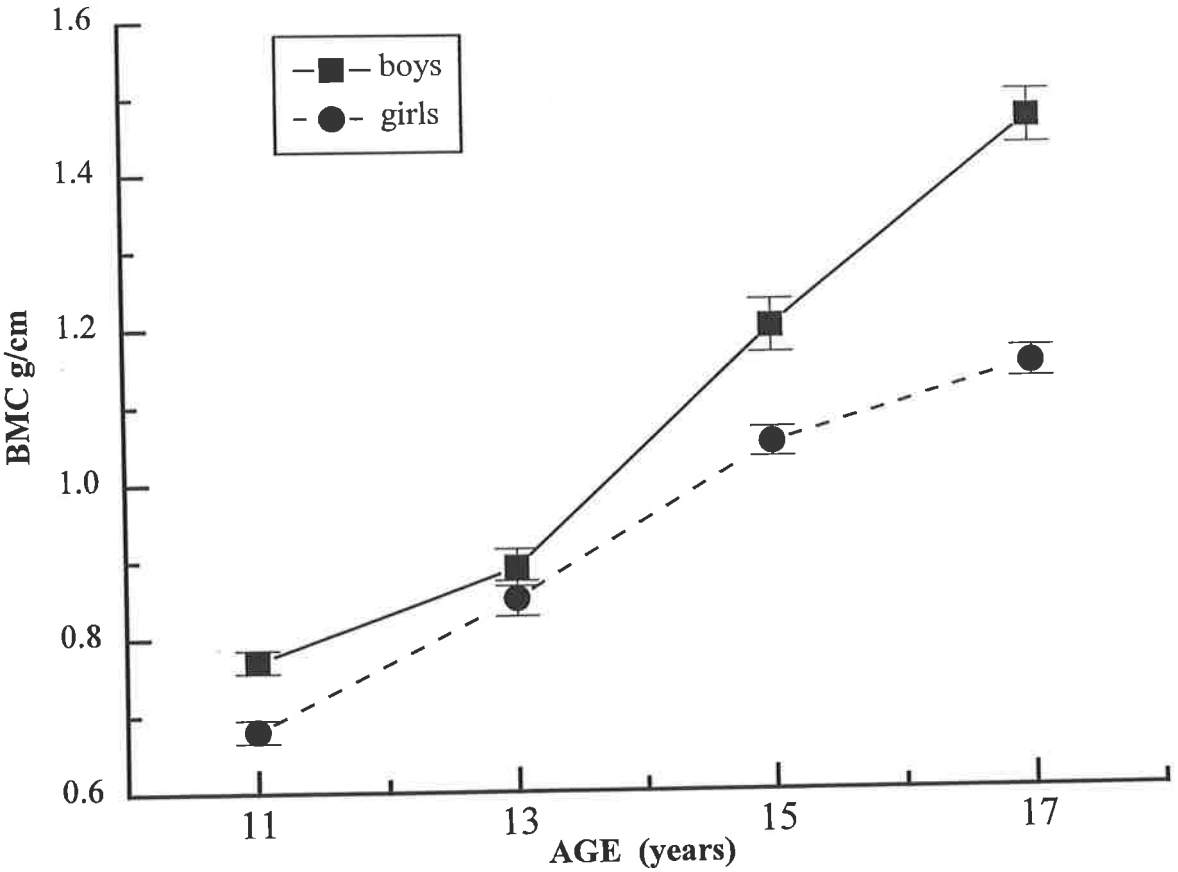


Figure 4.1.1 BMC by age and sex (mean ± sem)

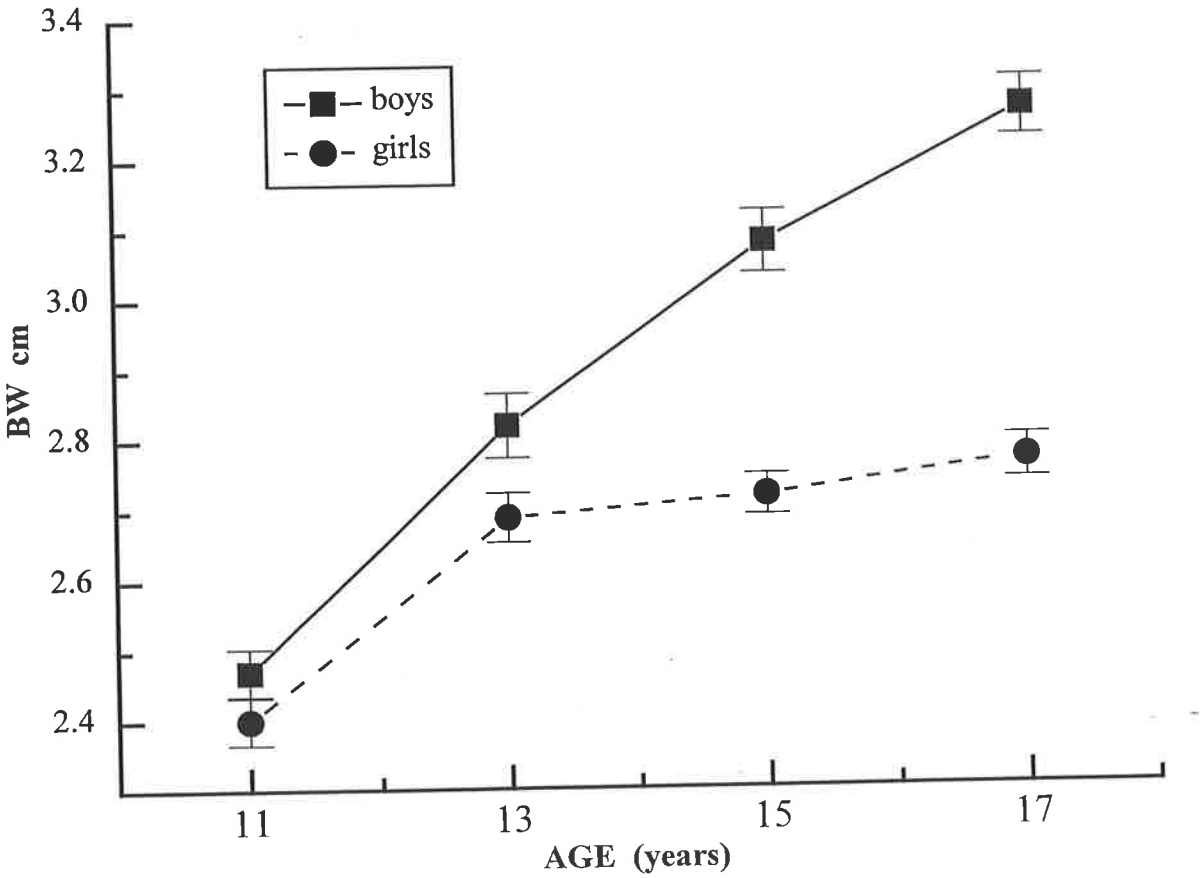


Figure 4.1.2 BW by age and sex (mean ± sem)

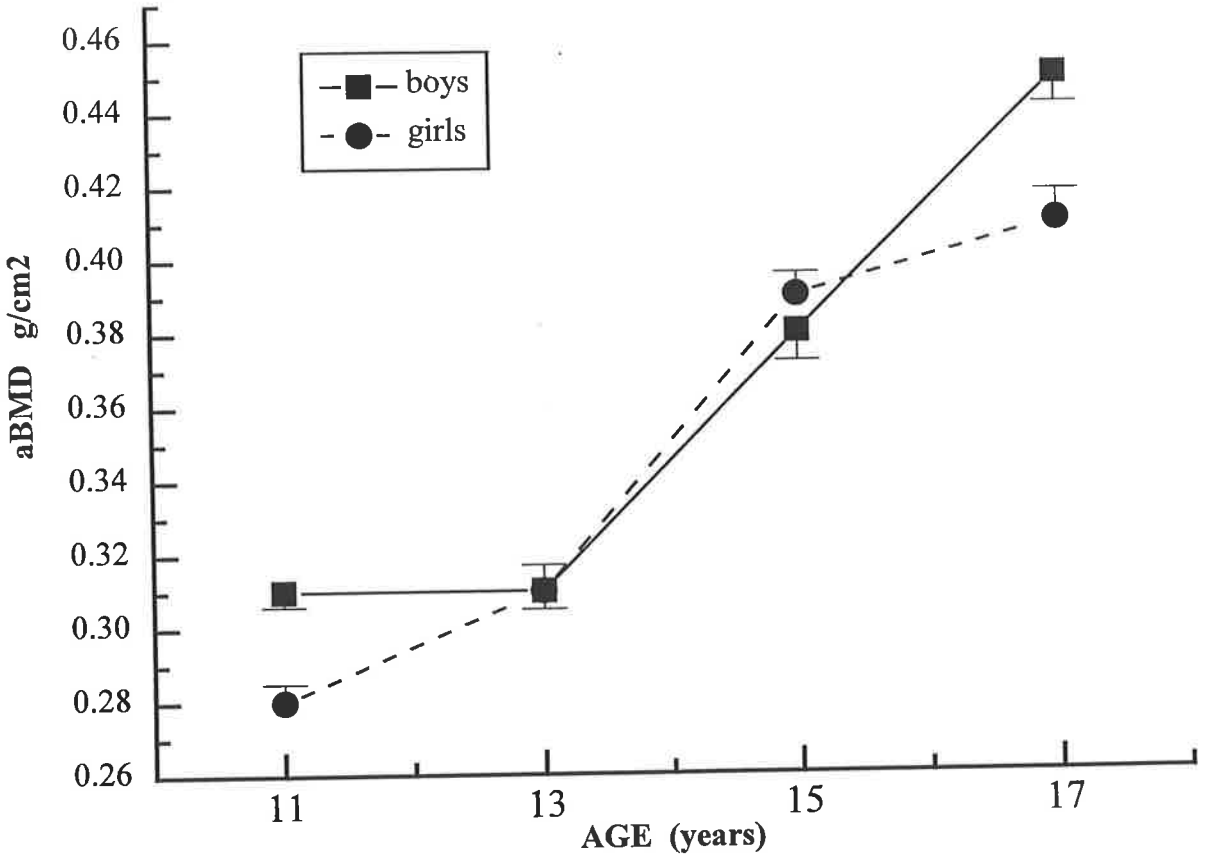


Figure 4.1.3 aBMD by age and sex (mean ± sem)

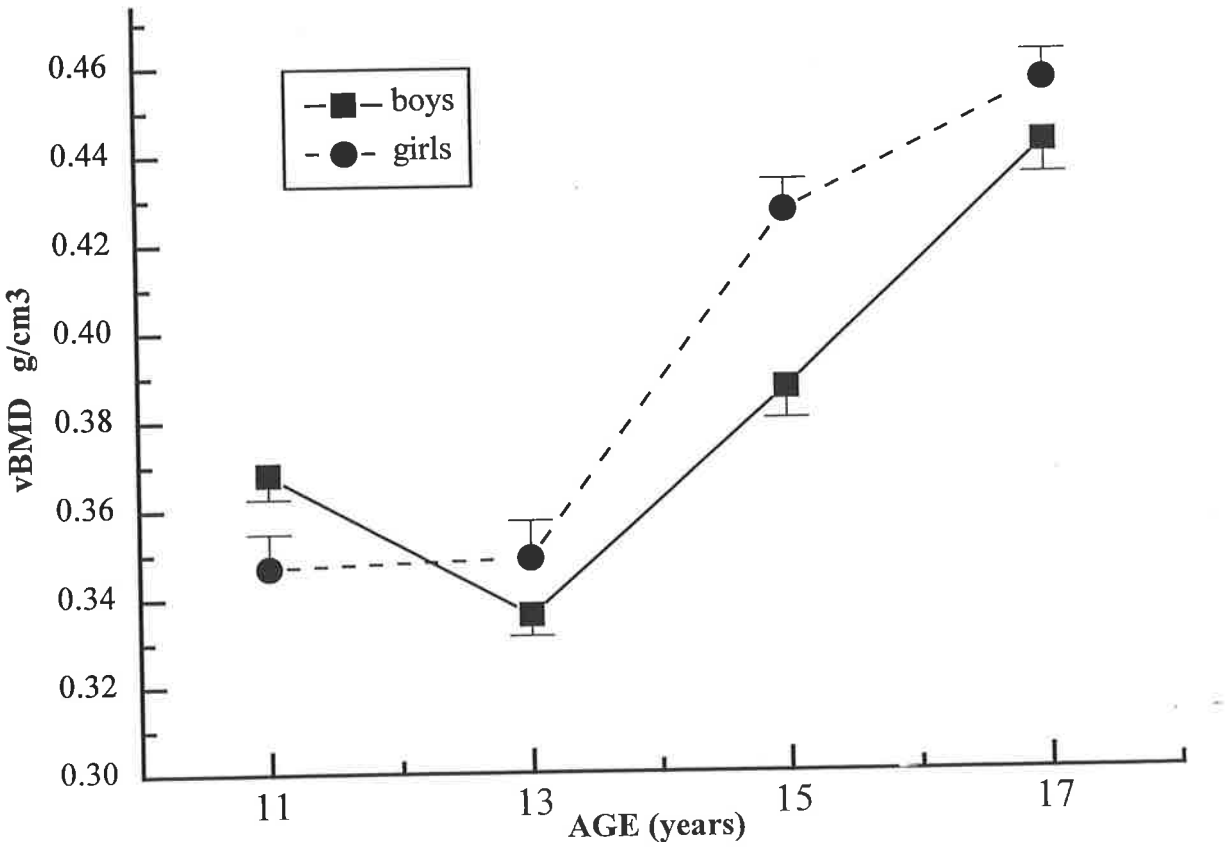


Figure 4.1.4 vBMD by age and sex (mean ± sem)

Two factor repeated measures analysis of variance was undertaken to investigate the changes in bone variables over time. Repeated measures investigate differences within groups ie time effects, differences between groups (gender effects) and interactions between the two. Table 4.1.2 presents the F values and significance for each of these differences for each bone variable.

Table 4.1.2

F statistic and probability of effects of time and gender and interaction of the two on each bone variable.

Variable	Time	Gender	Time x Gender
BMC	968	20	54
BW	511	27	74
aBMD	624	3.4[a]	14
vBMD	281	0.4[a]	9.4

$p<0.001$ except a: not significant

For all four bone variables there was a significant time effect. Post-hoc testing for BMC showed that BMC increased at each age in both boys and girls. BMC at 13 years was significantly greater than at 11 years, BMC at 15 years was significantly greater than at 13 years, and BMC at 17 years was significantly greater than at 15 years. For BW this was also the case in boys but in girls the only significant increase was in the two year interval 11 to 13 years, although in the four year interval 13 to 17 years, the increase was also significant. In boys aBMD was no different between ages 11 and 13 but increased significantly after that at each age. In girls, there were no differences between 11 and 13, and 15 and 17 years, but a significant increase occurred from 13 to 15 years. vBMD decreased in boys from age 11 to age 13 years then increased significantly at each subsequent age. In girls, vBMD was no different between 11 and 13 years, but increased significantly at both 15 and 17 years.

There was a significant gender effect in both BMC and BW (Table 4.1.2). Post-hoc testing indicated that BMC and BW were significantly greater in boys than girls at 15 and 17 years. Although there was no significant gender effect on aBMD and vBMD across time, post-hoc

testing showed that aBMD was significantly greater in boys than girls at 17 years and vBMD was significantly greater in boys than girls at 11 years and significantly lower at 15 years.

These gender differences in bone variables at different ages were confirmed by the highly significant interaction of time and gender (Table 4.1.2). All four bone variables increased with age, but the effect of time was different in boys and girls.

A test of goodness of fit of the regression of each bone variable on age, in each sex, indicated that there was a linear relationship between age and every bone variable ($p<0.001$ to reject the hypothesis that there is no linear relationship).

4.1.2 Tracking

Tracking (or canalisation) describes the consistency, through time, of biological variables ie the tendency of individuals to maintain their ranked position in the distribution curve through time. Tracking is here defined by the Pearson correlation coefficient between age points. Table 4.1.3 presents the tracking coefficients for each of the four bone variables in boys and girls.

There was a high level of tracking for all four bone variables between most ages. In boys, the tracking coefficients for BMC varied from 0.70 to 0.95 with significantly higher values at older ages (13-15, 13-17, 15-17). BW followed a similar level of tracking but with less variation. The tracking coefficient for aBMD was significantly less than those for BMC and BW except in the interval 15 - 17 years when it was as great as for BMC and BW and significantly greater than that for vBMD at all other intervals. The tracking coefficient for vBMD was highest in the interval 15-17 years and significantly higher then than for all other intervals. For most intervals, the tracking coefficient of vBMD was significantly less than that of BMC and BW. In girls, the tracking coefficient was significantly higher in the 2 year intervals than in the 4 and 6 year intervals and highest at 15 to 17 years. The tracking coefficient of aBMD was no different from the tracking of BMC and BW at any interval except 11-13 years. The tracking coefficient of vBMD was similar at each interval but in most cases significantly less than that of BMC and BW but no different from that of aBMD.
Comparison of tracking in boys and girls indicated that the coefficients were not significantly different for any bone variable and any interval except for vBMD 13-17 years, the tracking coefficient of which was significantly higher in girls.

The tracking coefficients of the bone variables were comparable with those of height over similar intervals eg boys: 11-13 years 0.91; 11-17 years 0.78; girls: 11-13 years 0.86; 11-17 years 0.60.

Examination of the variance of each bone variable indicated that it was largest in BMC in boys (14-21%) and smallest in BW in girls (7-9%). In both boys and girls the largest variance was in aBMD. For each bone variable, the greatest variance occurred in the age range 13 to 15 years in boys and 11 to 15 years in girls.

Table 4.1.3
Matrix of tracking coefficients of BMC (top line), BW (second line), aBMD (third line), and vBMD (fourth line), between age points in boys to the right and girls to the left of the diagonal. (n = boys 55, 55, 51; girls 48, 48, 42; at 13, 15 and 17 respectively).

Age years	11	13	15	17
11		0.80 0.81 0.65 0.54	0.70 0.82 0.48 0.34[b]	0.78 0.85 0.50 0.35[b]
13	0.90 0.82 0.74 0.57		0.92 0.88 0.72 0.39[a]	0.91 0.77 0.67 0.19[c]
15	0.82 0.71 0.60 0.46	0.92 0.83 0.88 0.58		0.95 0.88 0.90 0.81
17	0.69 0.74 0.61 0.58	0.73 0.83 0.70 0.70	0.91 0.93 0.85 0.79	

$p<0.001$ except a: $p<0.005$, b: $p<0.01$, c: not significant

4.1.3 Rate of change in bone status

Tables 4.1.4 and 4.1.5 present the absolute changes in each bone variable for each two-year interval and in the six year interval 11 to 17 years, and the change expressed as a percentage of the value at the younger age, in boys and girls respectively. Figures 4.1.5 to 4.1.8 present the percentage changes in each bone variable comparing boys and girls, and Figures 4.1.9 and 4.1.10 present the data comparing all four bone variables in boys and girls respectively.

Table 4.1.4
Mean, standard deviation (sd) and range of the absolute and percentage change in each bone variable in each of the 2 year intervals and the 6 year interval, in boys.

Interval n	11-13y 54	13-15y 54	15-17y 51	11-17y 51
Absolute change				
ΔBMC g/cm mean : sd range	0.12 : 0.10 -0.08 - 0.44	0.30 : 0.12 0.07 - 0.53	0.28 : 0.08 0.08 - 0.51	0.71 : 0.06 0.34 - 1.15
ΔBW cm mean range	0.35 : 0.19 -0.05 - 0.80	0.27 : 0.16 -0.04 - 0.61	0.19 : 0.15 -0.13 - 0.55	0.79 : 0.16 0.48 - 1.10
ΔaBMD g/cm^2 mean : sd range	0.004 : 0.025 -0.07- 0.05	0.07 : 0.04 -0.002- 0.15	0.06 : 0.02 0.01 - 0.14	0.14 : 0.05 0.04 - 0.22
ΔvBMD g/cm^3 mean : sd range	-0.03 : 0.035 -0.13 - 0.03	0.05 : 0.05 -0.05 - 0.14	0.05 : 0.03 0 - 0.14	0.07 : 0.05 -0.07 - 0.18
Percentage change				
ΔBMC mean : sd range	16 : 12 -9 - 52	34 : 12 8 - 65	25 : 10 6 - 46	93 : 21 48 - 136
ΔBW mean : sd range	14 : 8 -2 - 36	10 : 6 -1 - 23	6 : 5 -5 - 18	33 : 7 18 - 47
ΔaBMD mean : sd range	1 : 8 -18 - 17	22 : 13 -1 - 47	18 : 8 3 - 39	46 : 15 11 - 75
ΔvBMD mean : sd range	-8 : 9 -29 - 10	15 : 15 -13 - 46	14 : 9 0 - 38	20 : 15 -14 - 53

Table 4.1.5
Mean, standard deviation (sd) and range of absolute and percentage change in each bone variable in each of the 2 year intervals and the 6 year interval, in girls.

Interval n	11-13y 48	13-15y 47	15-17y 42	11-17y 42
Absolute change				
ΔBMC g/cm mean : sd range	0.16 : 0.07 0.01 - 0.33	0.21 : 0.06 0.09 - 0.33	0.09 : 0.06 -0.01 : 0.21	0.46 : 0.09 0.29 - 0.67
ΔBW cm mean range	0.29 : 0.13 0.06 - 0.49	0.04 : 0.12 -0.37 - 0.37	0.05 : 0.07 -0.10 - 0.25	0.37 : 0.15 0.01 - 0.69
ΔaBMD g/cm^2 mean : sd range	0.03 : 0.03 -0.03 - 0.10	0.07 : 0.02 0.02 - 0.12	0.03 : 0.02 -0.01 - 0.06	0.13 : 0.03 0.07 - 0.19
ΔvBMD g/cm^3 mean range	-0.001 :0.05 -0.11 - 0.09	0.08 : 0.03 0.01 - 0.17	0.03 : 0.03 -0.03 - 0.08	0.11 : 0.04 0.03 - 0.18
Percentage change				
ΔBMC mean range	23 : 10 2 - 47	27 : 10 9 - 48	9 : 6 -1 - 24	69 : 17 34 - 100
ΔBW mean range	12 : 6 2 - 23	2 : 5 -12 - 16	2 : 3 -4 - 9	16 : 7 0 - 33
ΔaBMD mean range	10 : 11 -12 - 35	25 : 9 5 - 44	7 : 6 -3 - 19	46 : 13 22 - 76
ΔvBMD mean range	0 : 13 -23 - 28	24 : 11 2 - 53	7 : 7 -6 - 20	33 : 16 6 - 64

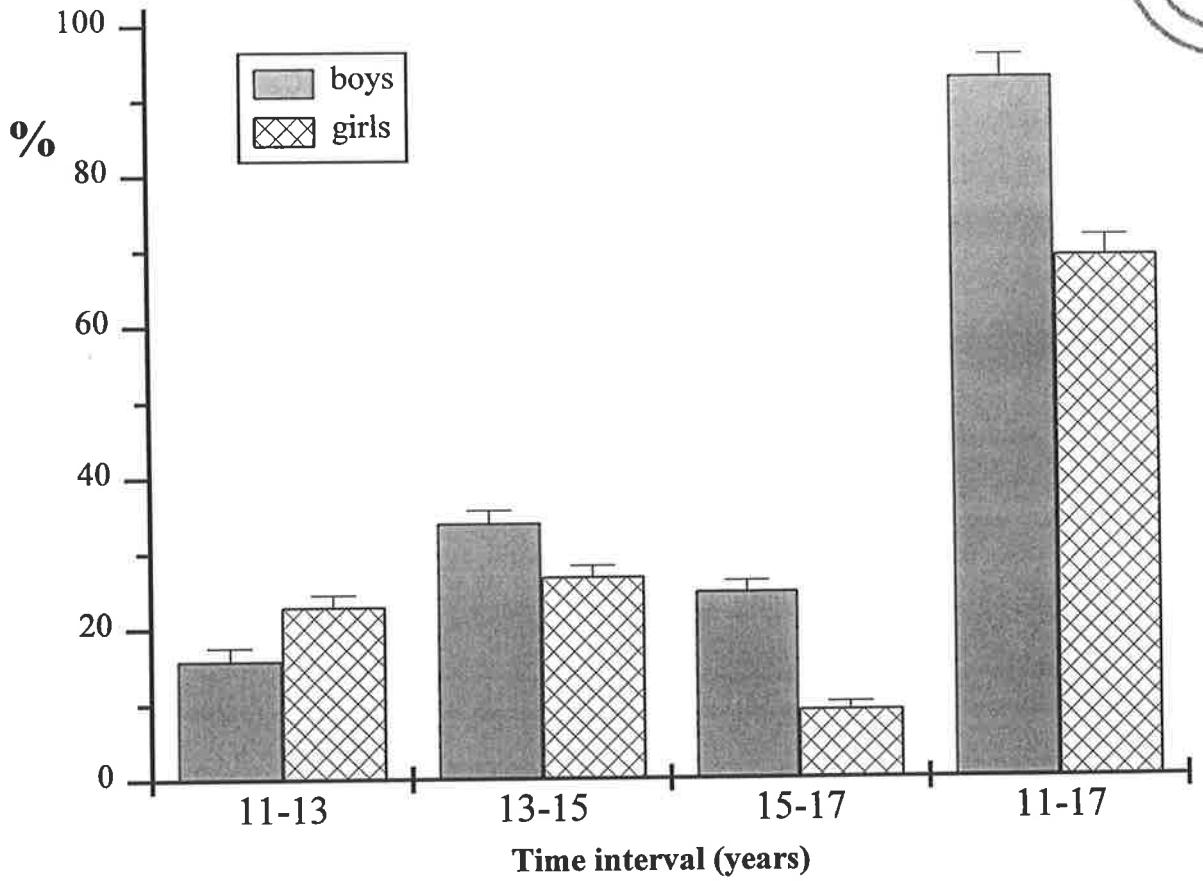


Figure 4.1.5 Percent change in BMC (mean + sem)

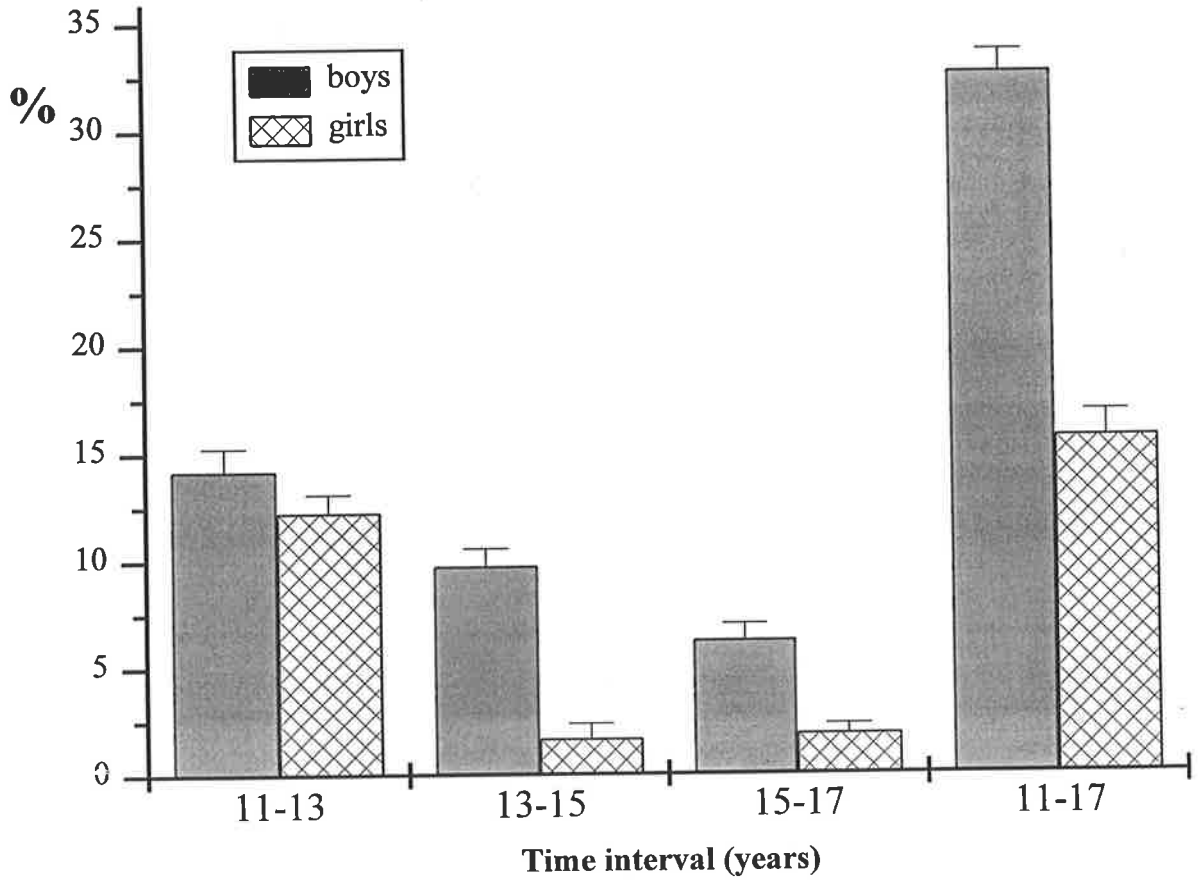


Figure 4.1.6 Percent change in BW (mean + sem)

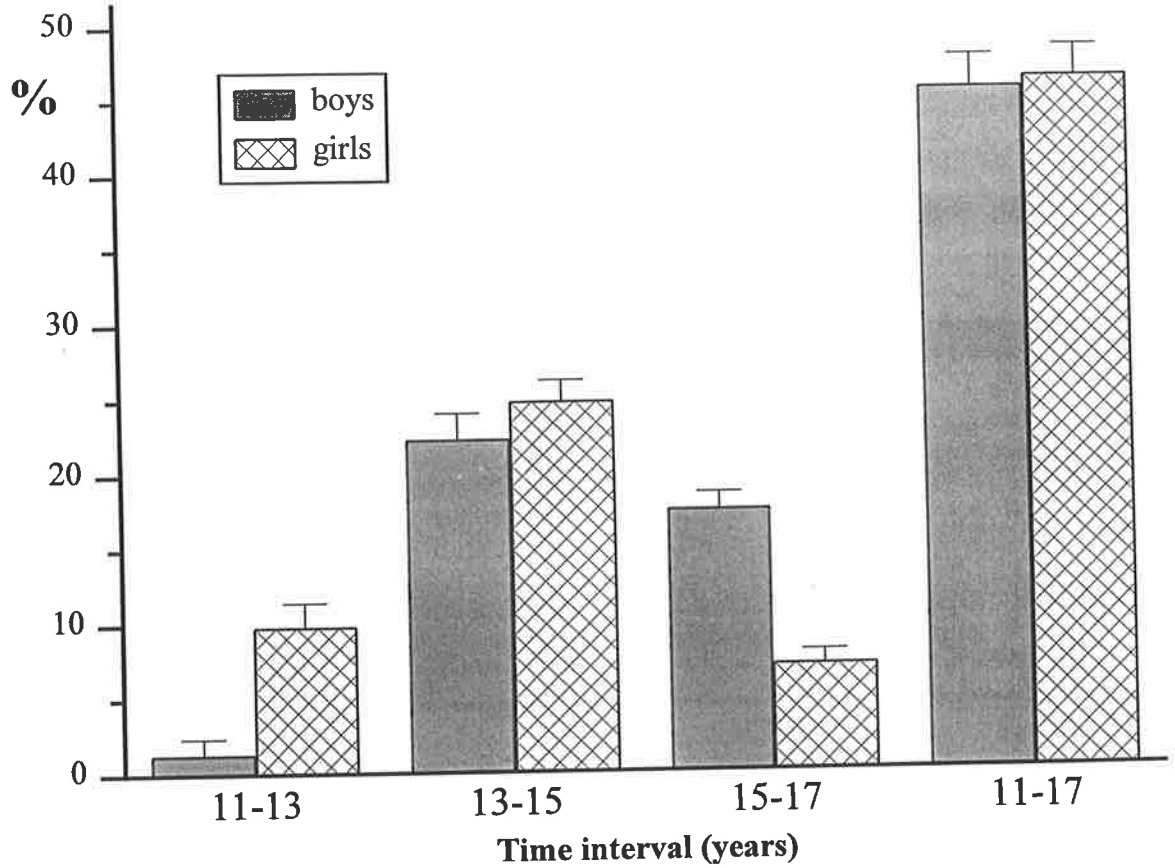


Figure 4.1.7 Percent change in aBMD (mean + sem)

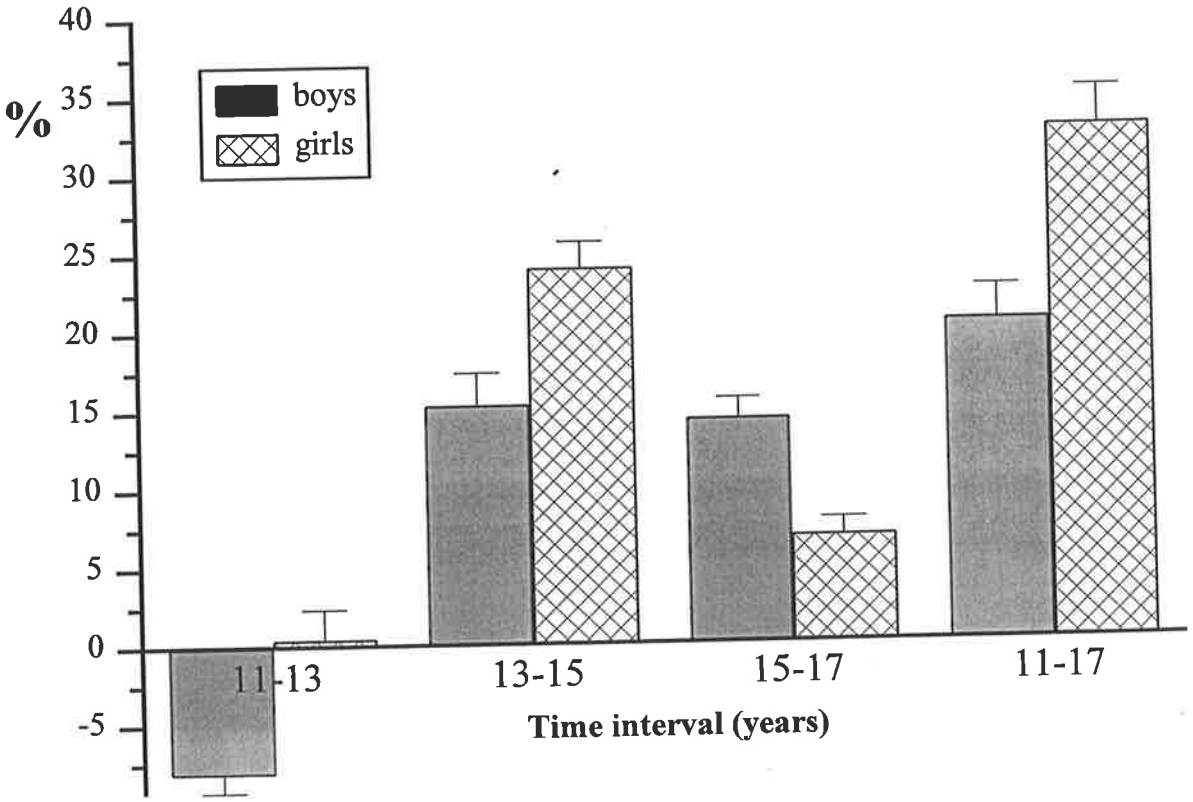


Figure 4.1.8 Percent change in vBMD (mean + sem)

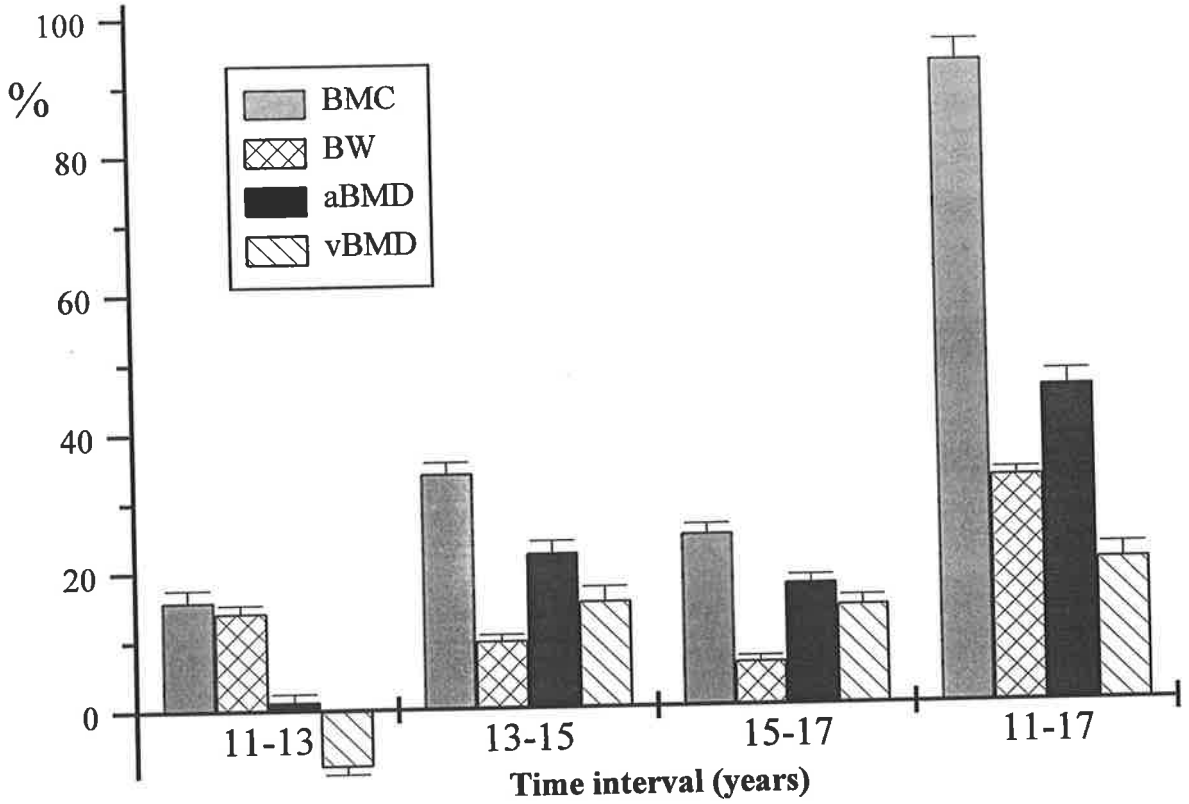


Figure 4.1.9 Percent change in each bone variable in boys (mean + sem)

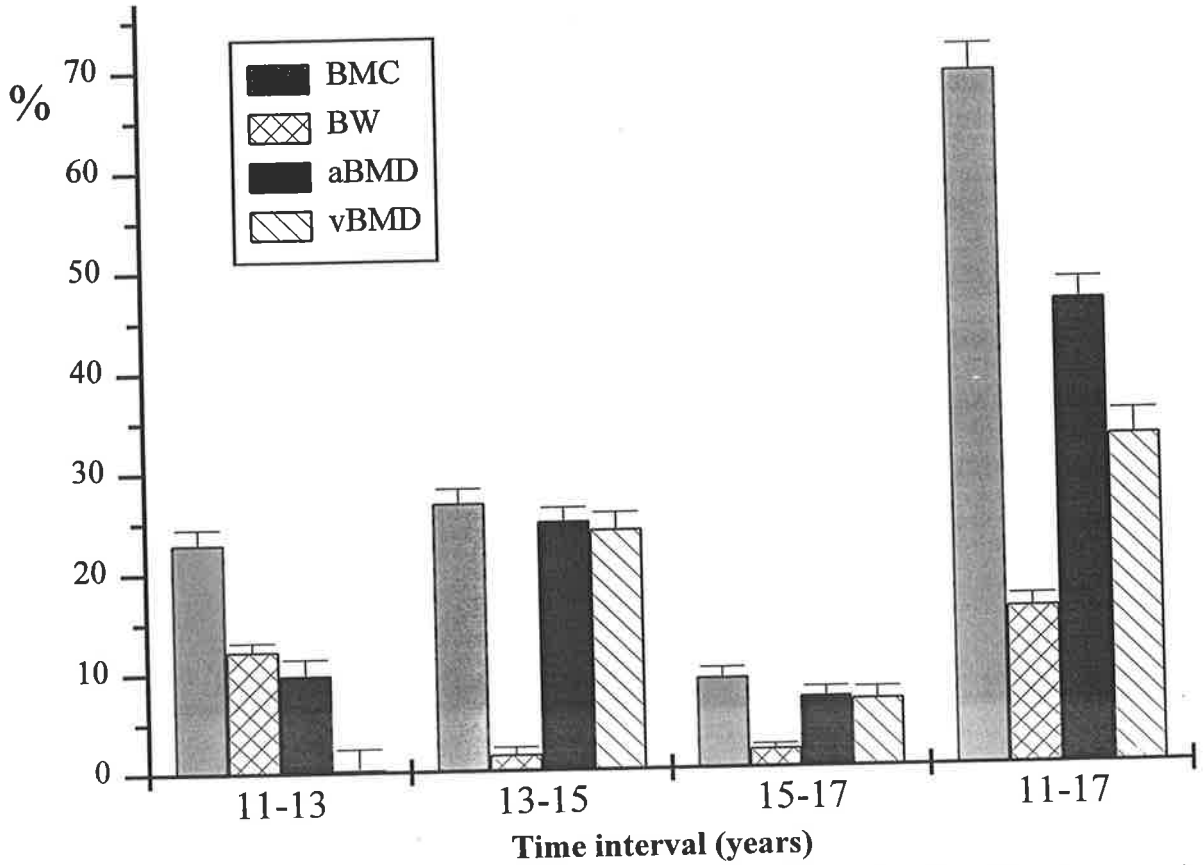


Figure 4.1.10 Percent change in each bone variable in girls (mean + sem)

Two factor repeated measures analysis of variance was undertaken to investigate differences in the rate of change over time. Table 4.1.6 presents the F values and significance of differences within groups (time effects), differences between groups (gender effects) and interactions between the two for the absolute change in each bone variable.

Table 4.1.6

F statistic and probability for time and gender effects and interaction of the two for absolute change in each bone variable.

Variable	Time	Gender	Time x Gender
ΔBMC	46	64	38
ΔBW	36	176	5.9[b]
ΔaBMD	80	1.5[c]	26
ΔvBMD	94	12	6.5[a]

$p<0.001$ except a: $p<0.01$, b: $p<0.05$, c: not significant

There was a significant time effect on the rate of change in all four bone variables. Post-hoc testing of BMC showed that in boys the change in the first two-year interval was less than the changes in both the second and third intervals. There was no difference between the changes in the second and third intervals. In girls the change in the first two-year interval was less than that in the second interval but greater than that in the third interval. For BW the change in the first interval was less than that in the next two intervals in both boys and girls, but in boys the change in the second interval was greater than that in the third interval, whilst there was no in girls. The change in aBMD in the second interval was greater than that in the first interval in both boys and girls. In boys there was no difference between the change in the second interval and that in the third but in girls the former was greater than the latter. There was no difference between the change in the first and third intervals in girls but in boys the change in the third interval was greater than that in the first interval. The pattern in vBMD was the same as that in aBMD in boys and the same in girls except that the change in vBMD in the third interval was greater than that in the first interval.

There were significant gender effects in BMC, BW and vBMD. Post-hoc testing indicated that the changes in both BMC and BW in the second two intervals were greater in boys than in girls. There was a smaller negative change in vBMD in girls than boys in the first interval, a greater change in girls in the second interval and a greater change in boys in the third interval. Although there was no significant gender effect across time in change in aBMD, post-hoc testing indicated that the change in the first interval was greater in girls and the change in the third interval was greater in boys.

These differences between the genders in changes in the bone variables at the three intervals are confirmed by the significant interaction effect of time and gender (Table 4.1.6). Thus maximal change occurs at different times in boys and girls.

The analysis was repeated using the percentage change in each bone variable for each interval. The results were similar.

4.1.4 Relation to adult values

BMC and vBMD values in boys and girls at each age were expressed as a percentage of the mean values in same sex adults: men aged 22 to 50 years (n=78) (Wishart et al 1995) and premenopausal women aged 18 to 56 (n=100) (Wishart et al 1990) as determined in the Department of Nuclear Medicine, The Royal Adelaide Hospital. Results are shown in Table 4.1.7.

In boys BMC at age 11 was 45% of the adult value and this had increased to 86% by age 17 years. In girls BMC at 11 years was 55% of the adult value and at 17 years 93%. At 15 years, 5% boys and 8% of girls had attained mean adult BMC and at 17 years these values had increased to 17% of boys and 28% girls. A further 18% of boys and 32% of girls had values above 90% of the respective adult mean value at 17 years.

Although boys had attained 71% of adult vBMD at 11 years this fell in the following measurement at 13 years but increased to 86% by age 17. Whilst girls had attained 71% of adult vBMD by age 11 this increased to be 94% at 17 years. By age 15 several girls had attained mean adult vBMD but no boys. By age 17, 4% boys and 28% girls had attained mean adult vBMD and a further 29% of boys and 39% of girls had values within 10% of this, whilst

values as low as 65% for boys and 72% for girls were recorded.

Mean values of BMC and vBMD at each age and in both boys and girls were significantly less than for the corresponding adult value.

Table 4.1.7
BMC (g/cm) and vBMD ($g/cm^{3)}$ of boys and girls at each age expressed as a percentage of the mean young adult value.

Age years	11	13	15	17	Adult mean
Boys					
BMC					
mean : sd	45 : 6	52 : 10	70 : 15	86 : 15	1.707
range	33 - 78	35 - 78	45 - 104	59 - 124	
vBMD					
mean : sd	71 : 8	65 : 6	75 : 10	86 : 10	0.516
range	59 - 91	54 -- 81	57 - 95	65 - 115	
Girls					
BMC					
mean : sd	55 : 8	68 : 12	85 : 11	93 : 10	1.175
range	40 - 75	46 - 91	61 - 107	74 - 114	
vBMD					
mean: sd	71 : 10	71 : 11	88 : 10	94 : 9	0.483
range	53 - 104	50 - 97	65 - 108	72 - 117	

4.2 PUBERTAL STATUS, AGE AT MENARCHE, SEX HORMONES

4.2.1 Absolute bone status

4.2.1.1 Pubertal status

Figure 4.2.1 shows the frequency distribution of pubertal status (PS) as determined by pubic hair stage (PHS), at each age for boys and girls. Girls exhibited more advanced development than boys at each age.

Figures 4.2.2 to 4.2.5 present the means and standard errors of the mean (sem) of BMC, BW, aBMD and vBMD according to PHS at each age in boys and girls. BMC and BW increased with increasing PHS and within each PHS group were greater in older children. There was no change in aBMD or vBMD between the early stages of puberty but differences occurred between the early and the later stages of puberty. In prepubertal children and in those at stage 2, aBMD and vBMD were lower in the older subjects at each stage but in those with the more advanced PHS, bone density was greater in the older subjects.

At any one age it was not possible to determine the relationship between PHS and the bone variables due to small numbers in some groups and lack of representation of all stages. To overcome this problem results of all four ages were combined. The mean, standard deviation (sd) and range of each bone variable by sex and PHS in all ages combined is presented in Table 4.2.1, and these data are also represented in Figures 4.2.2 to 4.2.5 as the means and sem.

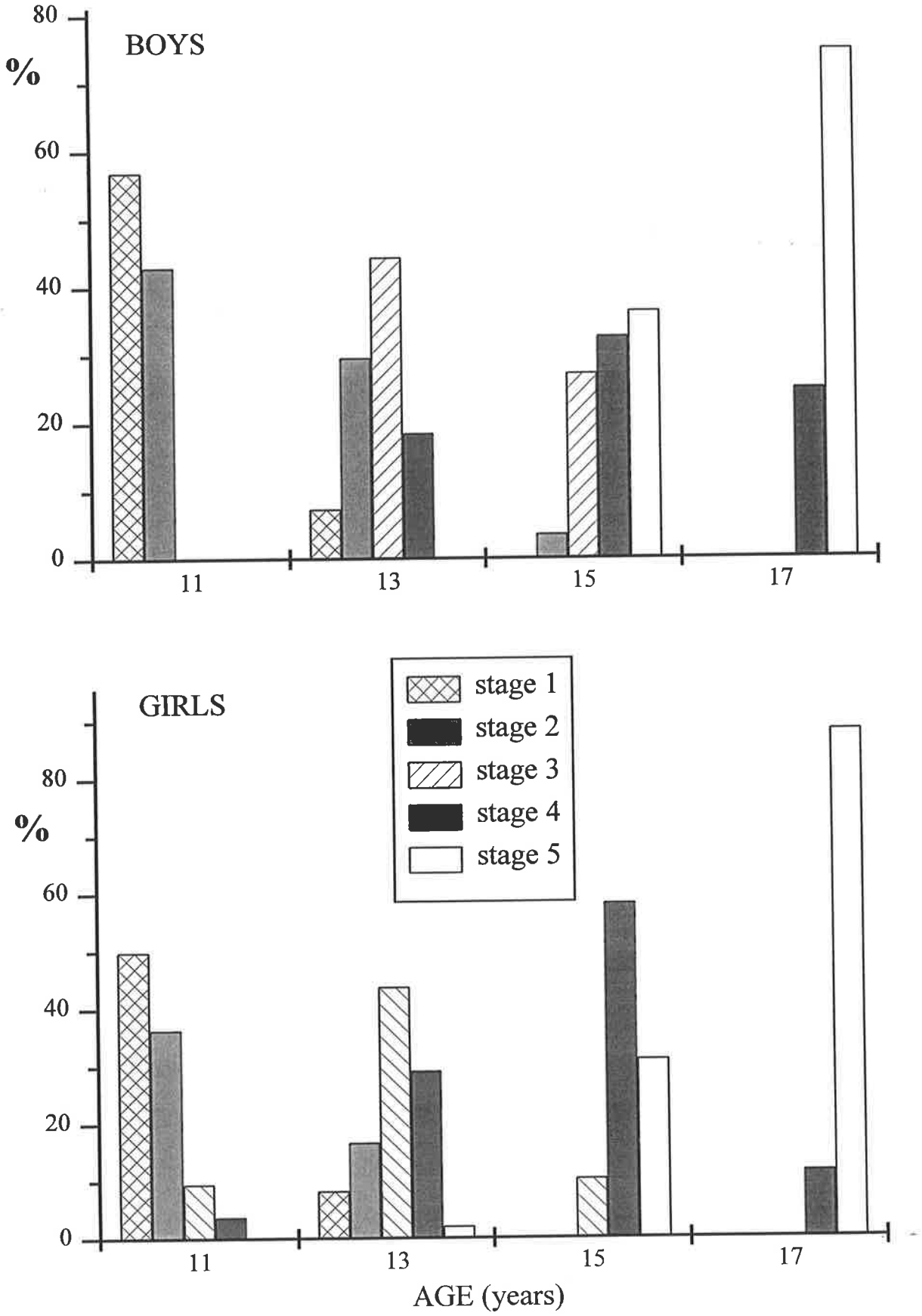


Figure 4.2.1 Frequency of pubic hair stage by age

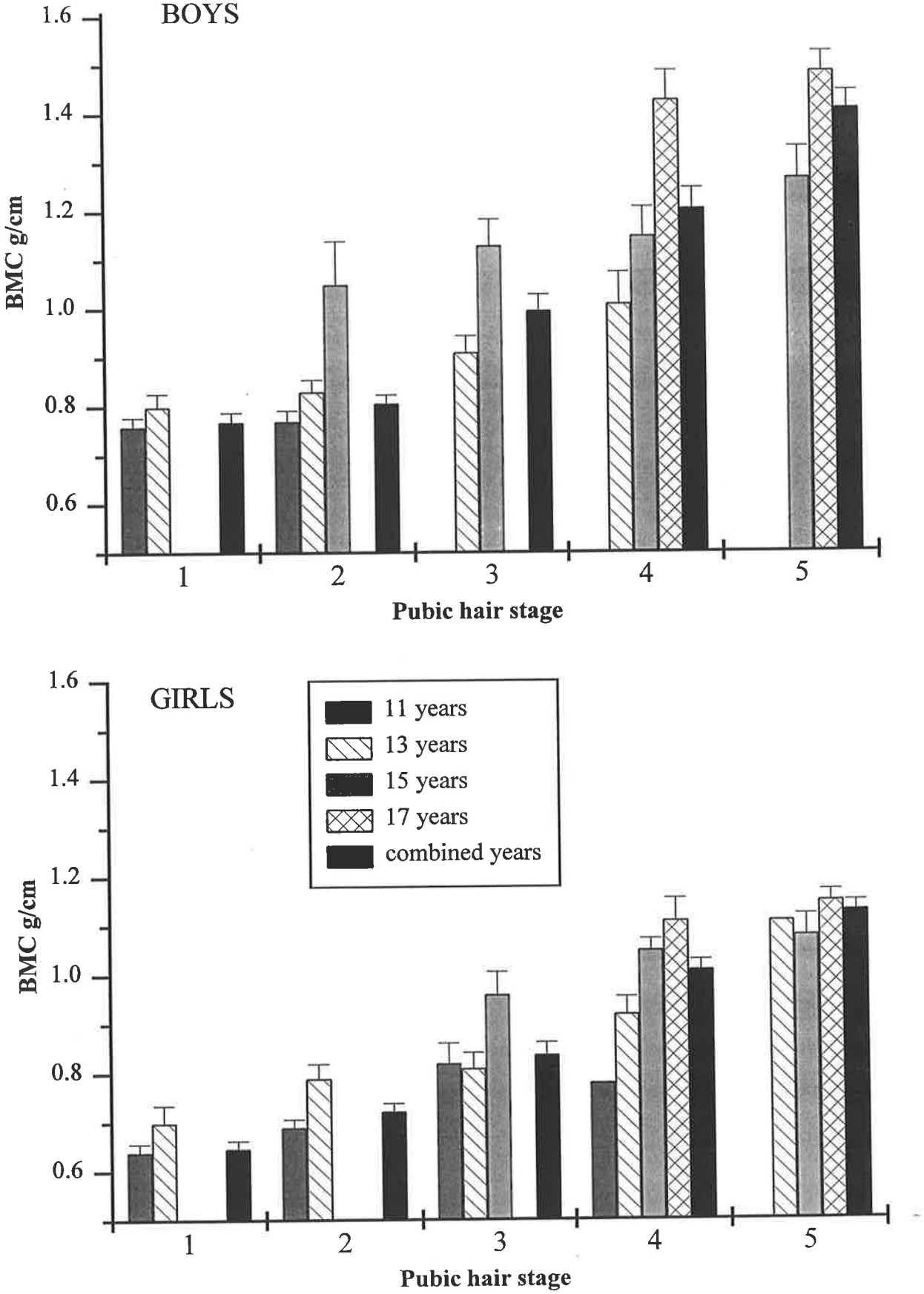


Figure 4.2.2 BMC according to PHS in boys and girls

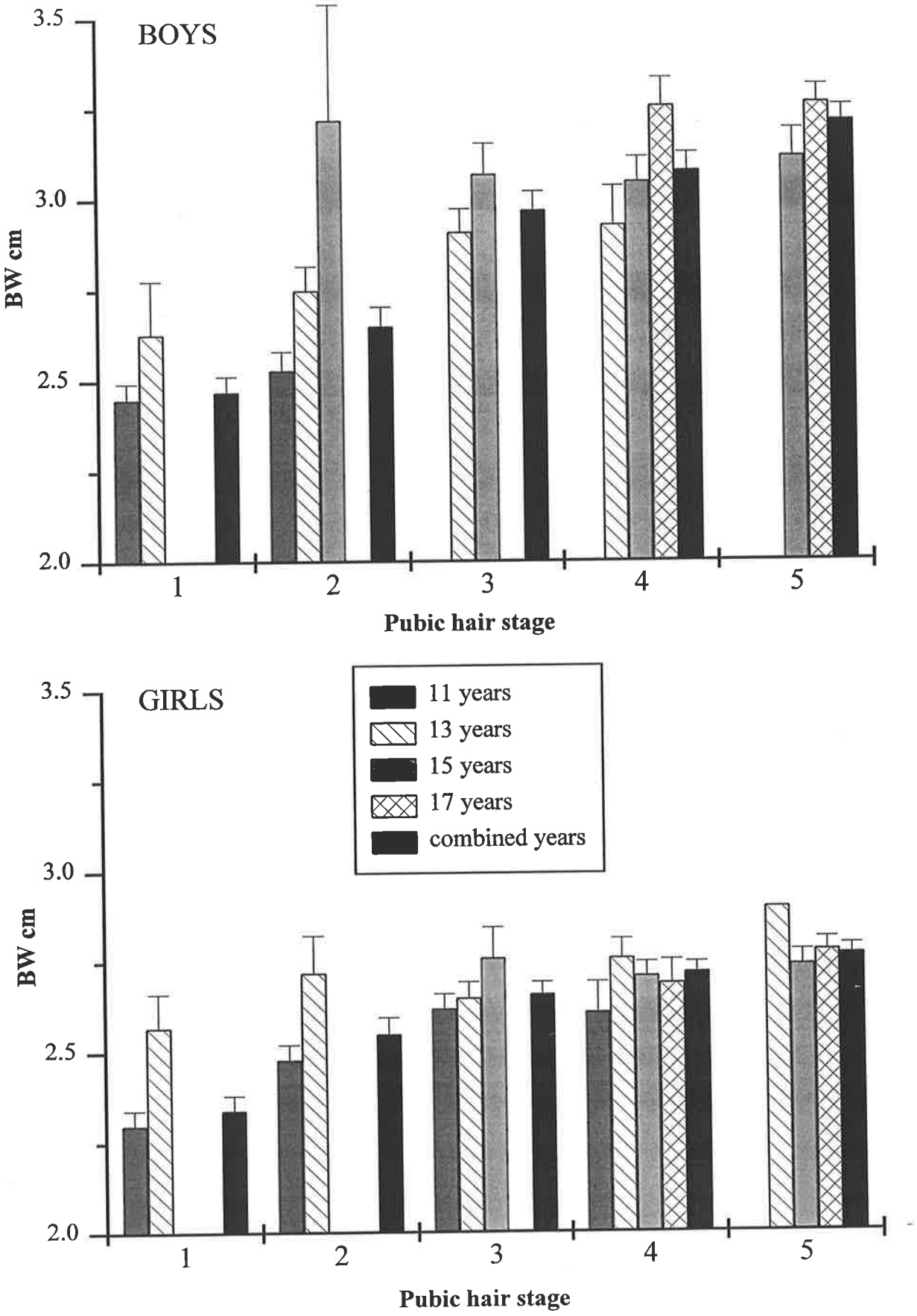


Figure 4.2.3 BW according to PHS in boys and girls

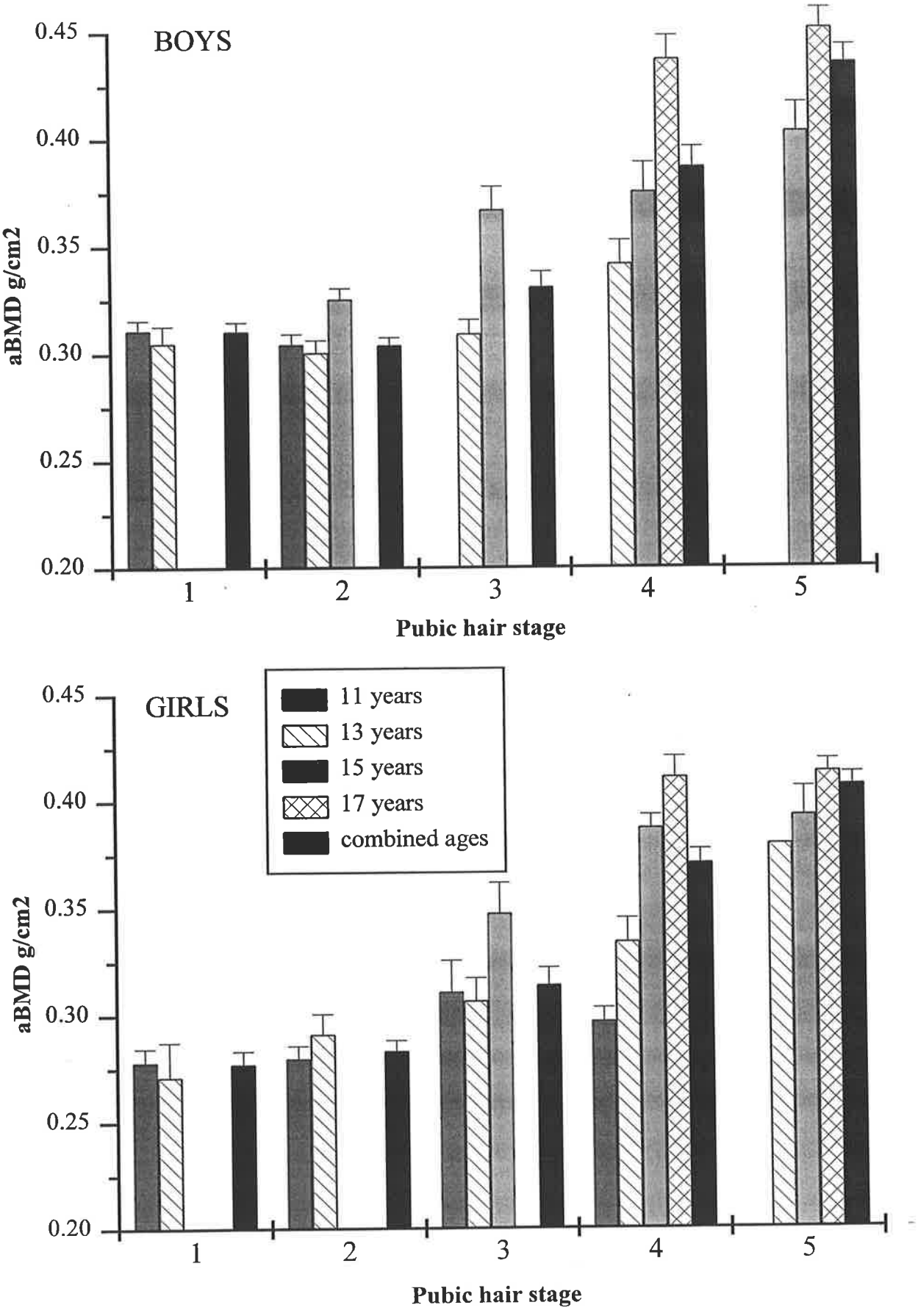


Figure 4.2.4 aBMD according to PHS in boys and girls

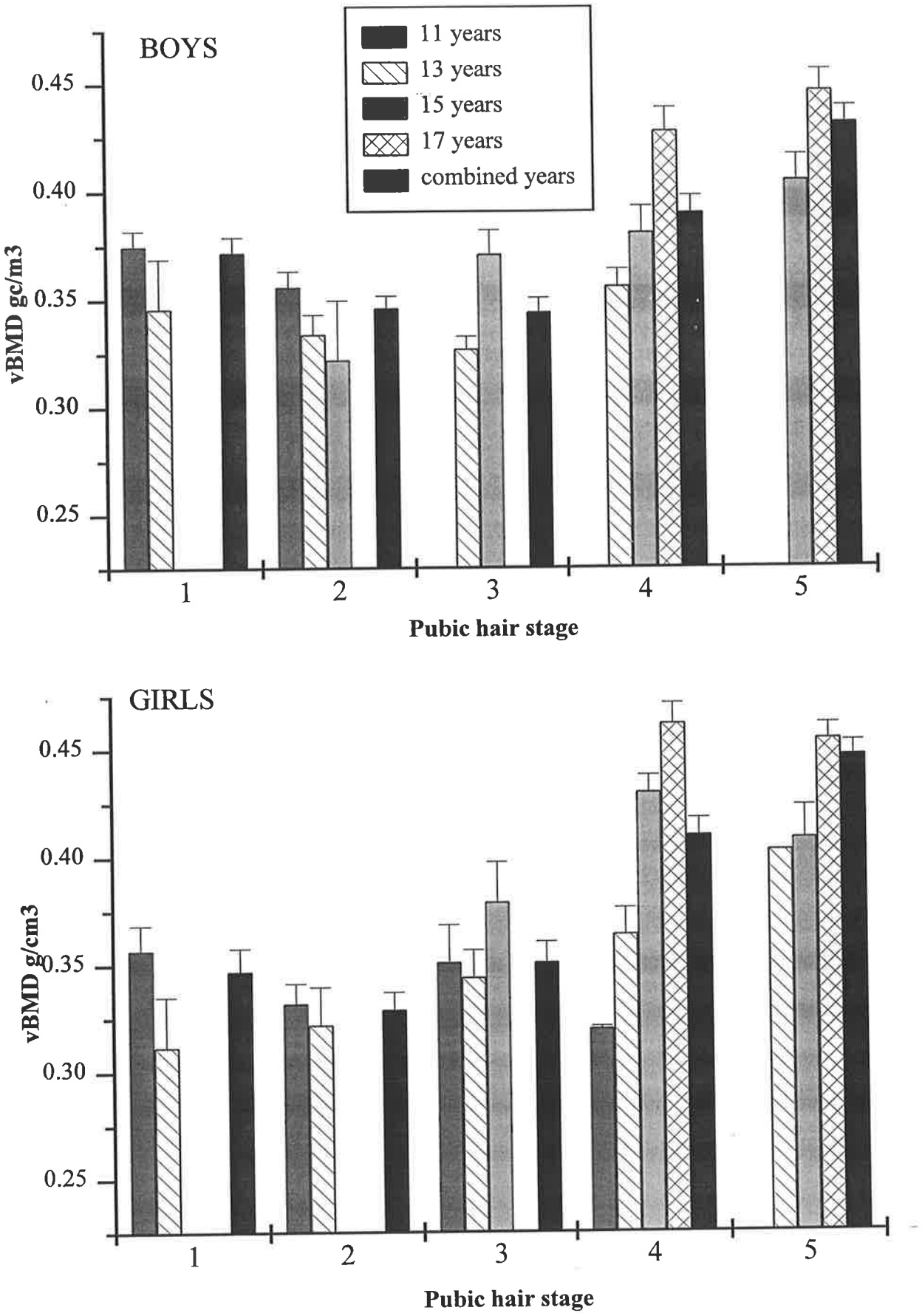


Figure 4.2.5 vBMD according to PHS in boys and girls

Table 4.2.1.
Mean, standard deviation (sd) and range in each of the bone variables in boys and girls, according to pubic hair stage (PHS).

PHS	1	2	3	4	5
Boys					
n	36	42	39	41	59
BMC g/cm					
mean :sd	0.77 : 0.10	0.81 : 0.13	1.00 : 0.21	1.21 : 0.28	1.41 :0.29
range	0.56 - 1.00	0.56 - 1.13	0.60 - 1.60	0.72 - 1.79	0.81 - 2.12
BW cm					
mean : sd	2.47 : 0.25	2.65 : 0.34	2.97 : 0.32	3.08 : 0.32	3.22 :0.32
range	2.04 - 3.01	1.97 - 3.56	2.26 - 3.71	2.54 - 3.85	2.53 - 3.92
aBMD g/cm^2					
mean :sd	0.31 : 0.02	0.30 :0.02	0.33 : 0.05	0.39 : 0.06	0.44 : 0.06
range	0.27 - 0.37	0.26 - 0.35	0.26 - 0.45	0.27 - 0.50	0.31 - 0.55
vBMD g/cm^3					
mean : sd	0.37 :0.04	0.35 : 0.04	0.34 : 0.04	0.39 : 0.05	0.43 : 0.06
range	0.29 - 0.47	0.28 - 0.46	0.29 - 0.43	0.30 - 0.50	0.32 - 0.59
Girls					
n	30	27	31	49	54
BMC g/cm					
mean : sd	0.65 : 0.08	0.72 : 0.09	0.84 : 0.14	1.01 : 0.14	1.13 : 0.14
range	0.49 - 0.86	0.60 - 0.89	0.56 - 1.12	0.63 - 1.29	0.76 - 1.40
BW cm					
mean : sd	2.34 : 0.22	2.55: 0.24	2.66:0.18	2.72 : 0.19	2.77 : 0.19
range	1.86 - 2.82	2.20 - 3.02	2.27 - 3.01	2.36 - 3.11	2.40 - 3.09
aBMD g/cm^2					
mean : sd	0.28 :0.03	0.28 :0.02	0.31 :0.05	0.37 : 0.04	0.41 :0.04
range	0.21 - 0.34	0.24 - 0.35	0.24 - 0.42	0.26 - 0.45	0.29 - 0.47
vBMD g/cm^3					
mean : sd	0.35 : 0.06	0.33 : 0.04	0.35 : 0.05	0.41 : 0.05	0.45 : 0.05
range	0.24 - 0.05	0.27 - 0.41	0.26 - 0.47	0.03 - 0.50	0.32 - 0.57

Two factor analysis of variance was used to determine the effect of gender and PS on bone variables. This analysis investigates differences within groups (pubertal status effect), differences between sexes (gender effects), and the interaction between the two. Table 4.2.2 presents the F values and significance for each of these differences for each bone variable.

Table 4.2.2

F statistic and probability of effects of pubertal status as PHS and gender, and interaction of the two on each bone variable.

Variable	PS	Gender	PS x Gender
BMC	146	100	3.8[a]
BW	66	123	6.5
aBMD	146	28	0.6[c]
vBMD	66	0.4[c]	3.1[b]

$p<0.001$ except a: $p<0.005$, b: $p<0.05$, c: not significant

There was a highly significant effect of PS on all four bone variables. Post-hoc testing showed that BMC was significantly greater at each consecutive stage except between stages 1 and 2 in both boys and girls. In boys, BW was greater at each of stages 3 to 5 than at stages 1 and 2 and greater at stage 5 than at stage 3. In girls BW at stage 1 was less than at all other stages, and at stage 2 was less than at stages 4 and 5. aBMD in boys was less at stages 1, 2 and 3 than at stages 4 and 5 and less at stage 4 than at stage 5. aBMD in girls was greater at each of stages 3, 4 and 5 than all preceding stages. In both boys and girls, vBMD at stages 2 and 3 was less than at stages 4 and 5 and at stages 1 and 4 was less than at stage 5.

There was a significant gender effect on BMC, BW and aBMD, but not on vBMD. Post-hoc testing indicated that BMC was greater in boys than girls at all stages; BW was greater in boys at all stages except stage 2; aBMD was greater in boys at stages 1, 2 and 5. There was a significant interaction effect between PS and gender on BMC and BW only (Table 4.2.2).

To determine the effect of age, gender and PS on bone variables a three factor analysis of variance was used. Due to the large number of empty cells PHS stages 1 and 2 were combined

and stages 4 and 5 were combined to reduce PHS to 3 categories. The F values and probability for each factor are presented in Table 4.2.3. There were significant effects of all three variables on BMC and BW. There were significant effects of gender and pubertal status but not of age on vBMD, and on aBMD there were significant effects of pubertal status and age but not of gender. There were no significant interaction effects of age, gender and pubertal status on any of the bone variables.

Table 4.2.3

F statistic and probability of effects of pubertal status as PHS, gender and age on each bone variable.

Variable	Age	PS	Gender
BMC	27.6	21.3	7.3[a]
BW	12.8	7.2	21.7
aBMD	17.3	16.8	0.02[c]
vBMD	2.6[c]	4.1[b]	8.8[a]

$p<0.001$ except a: $p<0.005$, b: $p<0.05$, c: not significant.

4.2.1.2 Age at menarche

The age at menarche ranged from 10.5 to 14.7 years, median 12.9 years, mean $\pm$ sd: 12.9 ± 1.2 years. At the 13 year visit, 18 (38%) girls had not reached menarche. By age 15 years only two girls had not reached menarche. At 17 years, one of these girls did not participate and the other had not reached menarche. There was no evident medical reason for this.

Bone variables at 13 years were compared between girls who had reached menarche and those who had not at the time of measurement. The mean values in the two groups and the significance of the differences are shown in Table 4.2.4. Those who had reached menarche had significantly greater BMC, BW and aBMD than those who had not but there was no difference in vBMD.

Table 4.2.4

Mean bone variables in pre- and post-menarchic girls at age 13 years and probability of T statistic for difference between the two groups.

Status	BMC g/cm	BW cm	aBMD g/cm^2	vBMD g/cm^3
Pre-menarche n= 18	0.74	2.60	0.28	0.33
Menarche n= 29	0.90	2.75	0.33	0.36
Probability	<0.001	0.02	0.001	0.10

The sample was further divided into four groups according to whether the subjects were one or more years pre-menarche (group 1), less than one year pre-menarche (group 2), had reached menarche in the last year (group 3), or were one or more years post-menarche (group 4). Analysis of variance was performed for each of the bone variables at 13 years, and the results are shown in Table 4.2.5. This showed significant differences in all bone variables between subjects grouped according to time from menarche (TM). Results of post-hoc testing to determine significantly different pairs are also shown in Table 4.2.5. BMC was greatest in those more than one year from menarche and significantly greater than those who were pre-menarchic. BW was least in those more than one year pre-menarchic and significantly less than the other three groups although there was no difference between these three groups. aBMD of those more than one year post-menarche was significantly greater than in the other three groups, between which there were no differences. vBMD was greatest in those more than one year post-menarche and significantly greater than in those less than a year either pre- or post-menarche, but the difference did not reach significance in those more than one year pre-menarche.

Table 4.2 5
Mean values of bone variables in girls at age 13 years grouped according to time from menarche (TM) and F statistic and probability for difference across TM groups.

Group 1: one or more years pre-menarche; Group 2: less than one year pre-menarche; Group 3: one year or less post-menarche; Group 4: 1 to 2 years post-menarche.

a,b,c denote pairs of significantly different groups

TM Group	1	2	3	4	F	p
n	7	9	14	15		
mean TM (yrs)	-1.26	-0.49	0.46	1.76		
BMC g/cm	0.69^{ab}	0.79^{c}	0.84^{a}	0.95^{bc}	8.8	0.0001
BW cm	2.47^{abc}	2.73^{a}	2.80^{b}	2.71^{c}	5.5	0.003
aBMD g/cm^2	0.28^{a}	0.27^{b}	0.30^{c}	0.35^{abc}	8.7	0.0001
vBMD g/cm^3	0.34	0.32	0.32	0.39	7.1	0.001

A similar analysis was performed for the subjects at age 15 years, but omitting those two subjects who had not reached menarche (Table 4.2.6), and for subjects at age 17 years (Table 4.2.7). These showed that within 4 years of menarche there was a significant association between TM and BMC, aBMD and vBMD with those further from menarche having greater values than those within a year of menarche. There was no significant association between TM and BW. When all subjects were two or more years post-menarche, there were no significant associations between TM and any of the bone variables (Tables 4.2.7).

Table 4.2.6
Mean values of bone variables in girls at age 15 years grouped according to time from menarche (TM) and F statistic and probability for difference across TM groups.

Group 3: one year or less post-menarche; Group 4: 1 to 2 years post-menarche; Group 5: 2 to 3 years post-menarche; Group 6: 3 to 4 years post-menarche.

a,b,c denote pairs of significantly different groups.

TM Group	3	4	5	6	F	p
n	7	11	13	15		
mean TM (yrs)	0.74	1.46	2.45	3.74		
BMC g/cm	0.92^{ab}	1.03	1.08^{a}	1.11^{b}	4.5	0.01
BW cm	2.61	2.77	2.79	2.71	2.6	0.07
aBMD g/cm^2	0.35^{a}	0.37	0.39	0.41^{a}	5.0	0.005
vBMD g/cm^3	0.40^{a}	0.41^{b}	0.42	0.46^{ab}	3.9	0.01

Table 4.2.7
Mean values of bone variables at 17 years grouped according to time from menarche (TM) and F statistic and probability for difference across TM groups.

Group 5: 2 to 3 years post-menarche; Group 6: 3 to 4 years post-menarche; Group 7: 4 to 5 years post-menarche; Group 8: greater than 5 years post-menarche.

TM group	5	6	7	8	F	p
n	7	9	13	13		
mean TM (yrs)	2.72	3.47	4.44	5.8		
BMC g/cm	1.07	1.14	1.19	1.15	1.89	0.15
BW cm	2.64	2.82	2.86	2.74	2.35	0.09
aBMD g/cm^2	0.41	0.41	0.42	0.42	0.51	0.68
vBMD g/cm^3	0.47	0.44	0.45	0.47	1.31	0.28

4.2.1.3 *Sex hormones*

Table 4.2.8 presents the 10th, 50th and 90th percentile values of testosterone and oestradiol at 11, 13 and 15 years. Log transformation of the variables was required to correct for the skewness of the data.

Table 4.2.8

Serum testosterone and oestradiol levels at 11, 13 and 15 years.

Age years	n	Percentiles		
		10	50	90
Testosterone pmol/L				
11	33	0.10	0.30	1.4
13	42	0.42	5.90	21.0
15	53	3.8	17.8	31.3
Oestradiol pmol/L				
11	33	26	110	364
13	38	80	250	800
15	46	52	160	390

Oestradiol

Blood for estimation of oestradiol was not taken at any particular time of the menstrual cycle nor was the time in the cycle noted. Oestradiol levels increased with increasing pubertal stage at each age but the difference between pubertal stages was not significant by ANOVA. At 11 and 13 years there were significant differences between subjects grouped according to time from menarche ($p<0.005$, $p<0.01$ respectively). At 11 years subjects who had reached menarche had significantly higher levels than those subjects more than one year pre-menarche, and at 13 years subjects more than one year post menarche had higher oestradiol levels than those subjects more than one year premenarche. At 15 years when all subjects were menarchic there were no differences in oestradiol levels according to time from menarche. At 11 years there were significant positive associations between BMC, BW and aBMD and oestradiol ($p<0.001$, $p<0.001$, $p<0.05$ respectively). At 13 years there were weak positive associations

between oestradiol and BMC and aBMD and at 15 years with aBMD and vBMD ($p<0.05$ for each).

Testosterone

Testosterone levels were significantly positively associated with PHS at 13 years ($p<0.005$) when the range of stages was 1 to 4 but not at 11 years when subjects were at either stage 1 or 2, nor at 15 years when all but two subjects were at stage 3 or greater.

BMC and BW were significantly positively associated with testosterone levels ($p<0.02$) at 11 years, but aBMD and vBMD were not. At 13 years, there was a positive association between testosterone and BMC ($p<0.001$), BW ($p<0.001$) and aBMD ($p<0.05$) but not vBMD. At 15 years, there were significant positive correlations between testosterone levels and all bone variables ($p<0.002$). When subjects were divided according to quartiles of testosterone levels at 13 and 15 years, there were significant differences in BMC and BW across quartiles, but no differences in bone density (Table 4.2.9). A similar analysis at 15 years showed significant differences across quartiles of testosterone in all four bone variables (Table 4.2.9).

Table 4.2.9

Bone variables at 13 and 15 years according to quartiles of testosterone at 13 and 15 years respectively and F value and probability for ANOVA between quartiles

a,b,c denote pairs of significantly different groups

	Quartile				F	p
	1	2	3	4		
13 years						
Testosterone pmol/L	≤1.5	1.6 - 5.7	5.8 - 8.9	≥ 9.0		
n	10	11	10	11		
BMC g/cm	0.80^{a}	0.84^{b}	0.86^{c}	1.03^{abc}	4.7	0.007
BW cm	2.62^{a}	2.71^{b}	2.79	3.10^{ab}	5.9	0.002
aBMD g/cm^2	0.31	0.31	0.30	0.33	1.7	0.18
vBMD g/cm^3	0.35	0.34	0.33	0.33	0.81	0.49
15 years						
Testosterone pmol/L	≤8.4	8.5 - 16.8	16.9 - 25.8	≥ 25.9		
n	13	12	14	14		
BMC g/cm	0.95^{abc}	1.16^{a}	1.20^{bc}	1.40^{abc}	12.0	<0.001
BW cm	2.92^{a}	3.08	3.00^{b}	3.31^{ab}	4.6	0.006
aBMD g/cm^2	0.33^{ab}	0.38^{a}	0.40^{b}	0.42^{a}	12.5	<0.001
vBMD g/cm^3	0.34^{ab}	0.38	0.41^{a}	0.41^{b}	7.50	<0.001

4.2.2 Rate of change in bone status

4.2.2.1 Pubertal status

The relationship between the rate of change in bone variables and pubertal status as determined by PHS was investigated by analysis of variance. The change in each bone variable between 11 and 13 years was related to PHS at 11 years and a similar analysis for change between 13 and 15 years and PHS at 13 years.

In boys absolute and percentage changes in BMC and BW from 11 to 13 years were significantly greater in those who were at PHS stage 2 than in those who were at PHS stage 1 ($p<0.02$) at the time of the initial measurement, but changes in aBMD and vBMD were not. The small number of girls at PHS stage 4 at 11 years was combined with those at stage 3. Both absolute and percentage changes in all four bone variables were significantly associated with PHS at 11 years (Table 4.2.10). Changes in BMC, aBMD and vBMD increased with increasing maturity but change in BW decreased. For each variable, the change in those initially at stage 1 was different from that for those at stage 2 and 3-4.

Table 4.2.10

Percentage change in girls of bone variables between 11 and 13 years according to PHS at 11 years and F statistic and probability for difference between stages.

a,b,c denote pairs of significantly different groups

PHS 11 years	1	2	3 and 4	F	p
n	24	18	6		
ΔBMC	18.5^{ab}	27.2^{a}	28.0^{b}	5.3	0.009
ΔBW	15.2^{ab}	10.4^{a}	6.5^{b}	9.4	0.0004
ΔaBMD	3.0^{ab}	15.4^{a}	20.3^{b}	15.6	0.0000
ΔvBMD	-8.0^{ab}	6.8^{a}	15.3^{b}	18.6	0.0000

A similar trend was observed in boys for the change from 13 to 15 years and PHS at 13 years i.e. the change in BMC, aBMD and vBMD was greater as PHS increased, and the rate of change in BW decreased with increasing PHS. The association between PHS at 13 years and subsequent change was weakly significant in BMC, aBMD and vBMD ($p=0.04$), but not significant in BW, nor significant in any variable expressed as percentage change.

In girls there was a trend for the rate of change to decrease in all four bone variables from 13 to 15 years with increasing PHS at 13 years, but only the percentage change in BMC was significant ($p=0.03$). The changes from 15 to 17 years were not related PHS at 15 years but there was a general trend towards a lower rate of change in those at a more advanced stage of maturity at 15 years, in all bone variables in both sexes.

4.2.2.2 Age at menarche

The association between menarche and rate of change in bone variables was investigated by analysis of variance. Subjects were grouped, as previously presented, according to TM at 13 and 15 years and the relationship determined with bone mass change from 11 to 13 and 13 to 15 years respectively. Results are presented in Table 4.2.11. There were significant associations between TM and percentage change in all four bone variables at both time intervals.

From 11 to 13 years, the greatest change in BMC was in the two groups who were post menarche at 13 years, and the changes were significantly greater than in the premenarchic subjects. However from 13 to 15 years those subjects who were furthest post-menarche (Group 6) had a significantly smaller change in BMC compared with the other three groups. The greatest change in BW occurred around menarche, with those furthest from this point having the least change (Group 4, 11 to 13 years). The greatest change in aBMD occurred in post-menarchic subjects, and there was little change in premenarchic subjects. The greatest change in vBMD occurred in the first 3 years post menarche (Group 6 less than Groups 4 and 5 for 13 - 15 years). vBMD fell in premenarchic subjects and increased in those peri- and post-menarchic. Rate of change in vBMD was greatest in those 1 to 3 years postmenarche and least in those more than 3 years postmenarche (group 6).

Table 4.2.11
Percentage change in bone variables between 11 to 13 years and mean time from menarche (TM) at 11 and 13 years with subjects grouped according to TM at 13 years, and percentage change from 13 to 15 years with subjects grouped according to TM at 15 years, and F statistic and probability for difference between TM groups.

Group 1: one or more year pre-menarche; Group 2: less than one year pre-menarche;
Group 3: one year or less post-menarche; Group 4: 1 to 2 years post-menarche;
Group 5: 2 to 3 years post-menarche; Group 6: 3 to 4 years post-menarche.

a,b,c denote pairs of significantly different groups

Percent change 11 to 13 years						
TM Group at 13	1	2	3	4	F	p
N	7	9	14	15		
Mean TM 11y	-3.24	-2.52	-1.54	-0.26		
Mean TM 13y	-1.26	-0.49	0.46	1.76		
ΔBMC	12.9^{ab}	15.7^{cd}	29.2^{ac}	27.5^{bd}	11.1	0.0000
ΔBW	12.1	15.8^{a}	13.2	8.7^{a}	3.8	0.016
ΔaBMD	0.8^{ab}	-0.04^{cd}	14.4^{ac}	17.5^{bd}	14.1	0.0000
ΔvBMD	-7.5^{ab}	-11.4^{cd}	7.7^{ac}	12.6^{bd}	12.4	0.0000
Percent change 13 to 15 years						
TM Group at 15	3	4	5	6	F	p
N	7	11	13	14		
Mean TM 13y	-1.26	-0.40	0.58	1.81		
Mean TM 15y	0.74	1.54	2.58	3.79		
ΔBMC	32.8^{a}	31.9^{b}	29.6^{c}	17.6^{abc}	11.5	0.000
ΔBW	6.0^{ab}	1.7	-0.04^{a}	0.6^{b}	4.1	0.01
ΔaBMD	25.4	29.8^{a}	29.8^{b}	16.9^{ab}	8.5	0.0001
ΔvBMD	20.6	30.8^{a}	32.6^{b}	18.3^{ab}	5.5	0.003

4.2.2.3 Sex hormones

There were no significant correlations between oestradiol levels, at the initial or final age, and change in bone variables from 11 to 13 years, or between oestradiol at 15 years and the change from 15 to 17 years. However oestradiol at 15 years was significantly positively related with the changes in BMC, aBMD and vBMD from 13 to 15 years ($P<0.05$, 0.005, 0.005 respectively). A similar analysis of testosterone levels indicated only weak positive correlations between testosterone at 11 years and changes in aBMD and vBMD from 11 to 13 years, and testosterone at 13 years and change in aBMD from 13 to 15 years ($p<0.05$). There was no relation between change in testosterone and changes in any bone variable at either 11 to 13 years or 13 to 15 years.

4.3 ANTHROPOMETRIC MEASUREMENTS

The mean, sd and range of height, weight and skinfold thickness (sum of biceps, triceps, subscapular and suprailiac) at each age in boys and girls are shown in Table 4.3.1.
The standard deviation score (SDS) of the mean height and weight at each age is also shown. This is a measure of the difference of the sample from the international reference (Tanner and Davies 1985). Height and weight increased with increasing age in both sexes. The sample was taller and heavier than the international standard. There was little change in skinfold thickness with age in boys, but a consistent increase with age in girls.

4.3.1 Absolute bone status

The association between each of the anthropometric variables and the four bone variables was determined at each age for boys and girls. For this analysis log transformation of total skinfold was necessary to correct for the skewness of the data. Table 4.3.2 shows the univariate correlation coefficients for height, weight and skinfold thickness with each bone variable at each age and in the total sample incorporating measurements at the four ages. In boys BMC and BW were positively associated with height and weight at every age except 17 years. Results in aBMD and vBMD were variable. The strongest correlations between aBMD and height and weight occurred at 15 years. There were significant negative correlations between vBMD and height and weight at 11 years and maximal significant positive correlation with weight at 15 years.

In girls BMC and BW were positively related to height at each age except BMC at 15 years. Weight was positively related to BMC at 13 and 15 years and to BW at every age. The only significant correlations between height or weight and aBMD and vBMD, were between height and aBMD at 13 years, and a negative correlation between height and vBMD at 11 years.

Table 4.3.1
Mean, sd, range and 10, 50 and 90th percentiles of height, weight and total skinfold thickness in boys and girls at each age, and the standard deviation score (SDS) of the mean height and weight of the sample.

Age	n	Mean	SD	Range	Percentiles			SDS
					10	50	90	
Boys								
Height cm								
11	56	144.5	6.1	133.3-163.6	135.9	144.7	151.5	0.16
13	54	157.1	7.8	142.0-175.0	147.4	155.8	168.7	0.05
15	55	170.6	6.6	153.1-189.6	161.4	171.8	181.2	0.25
17	52	177.8	6.6	164.3-195.6	169.1	177.5	186.0	0.18
Weight kg								
11	56	38.4	7.0	26.3-56.9	29.5	37.1	48.5	0.35
13	54	48.5	10.4	29.7-71.3	35.0	47.4	63.6	0.30
15	55	61.0	11.1	40.1-82.3	46.0	59.6	78.1	0.38
17	52	72.0	9.9	46.8-96.9	59.7	72.6	84.0	0.33
Total skinfold mm								
11	56	34.9	14.9	15.6-78.4	20.8	31.4	56.1	
13	54	35.1	15.9	16.9-78.4	21.4	30.1	57.8	
15	55	35.4	20.0	16.0-131.9	21.0	28.9	64.1	
17	52	36.9	19.8	16.1-132.9	22.8	31.5	50.8	
Girls								
Height cm								
11	52	146.5	6.2	133.7-160.0	138.5	146.0	156.1	0.20
13	49	158.5	5.9	145.3-173.5	152.2	157.7	166.5	0.19
15	48	164.4	5.9	154.7-180.8	157.0	162.6	172.7	0.40
17	43	166.1	5.8	156.9-182.4	160.0	165.0	174.9	0.45
Weight kg								
11	52	40.3	7.8	25.7-59.0	29.7	40.9	52.1	0.32
13	49	52.1	10.7	32.1-82.7	37.8	51.6	68.0	0.49
15	48	58.1	11.2	42.0-94.3	44.7	56.4	73.1	0.38
17	43	63.9	12.7	45.9-100.0	50.7	62.3	86.2	0.58
Total skinfold mm								
11	52	44.9	19.9	23.5-93.2	24.7	37.9	78.4	
13	49	51.8	25.3	21.7-134.0	25.6	44.6	94.1	
15	48	53.1	25.3	21.7-134.0	27.4	48.4	100.5	
17	43	58.3	27.3	25.2-139.3	29.4	50.0	105.3	

Table 4.3.2

Univariate correlation coefficients between height, weight and total skinfold thickness and the four bone variables at each age and in all ages combined, in boys and girls.

Age years		11	13	15	17	all ages
Boys						
n		56	54	55	52	217
BMC	height	0.34[c]	0.51[d]	0.46[c]	0.05	0.77[d]
	weight	0.51[d]	0.58[d]	0.63[d]	0.36[c]	0.82[d]
	skinfold	0.16	0.07	0.13	0.03	0.05
BW	height	0.38[c]	0.54[d]	0.38[c]	0.20	0.82[d]
	weight	0.51[d]	0.61[d]	0.47[d]	0.43[d]	0.76[d]
	skinfold	0.19	0.17	0.05	0.09	0.07
aBMD	height	0.12	0.30[b]	0.39[c]	-0.10	0.70[d]
	weight	0.22	0.36[b]	0.57[d]	0.18	0.74[d]
	skinfold	0.04	-0.04	0.15	0.01	0.02
vBMD	height	-0.25[a]	-0.15	0.21	-0.20	0.40[d]
	weight	-0.25[a]	-0.17	0.37[c]	-0.04	0.43[d]
	skinfold	-0.17	-0.18	0.06	-0.01	-0.03
Girls						
n		52	48	48	43	191
BMC	height	0.44[d]	0.48[d]	0.20	0.41[c]	0.76[d]
	weight	0.20	0.32[a]	0.29[a]	0.23	0.76[d]
	skinfold	-0.11	0.17	0.21	0.17	0.22[c]
BW	height	0.59[d]	0.55[d]	0.33[b]	0.40[c]	0.69[d]
	weight	0.34[c]	0.43[d]	0.33[b]	0.30[a]	0.57[d]
	skinfold	0.13	0.35[b]	0.26[a]	0.35[a]	0.31[d]
aBMD	height	0.07	0.28[a]	0.02	0.20	0.65[d]
	weight	-0.04	0.15	0.13	0.03	0.53[d]
	skinfold	-0.27[a]	0.01	0.09	-0.08	0.13[a]
vBMD	height	-0.28[a]	0.05	-0.16	-0.04	0.42[d]
	weight	-0.21	-0.04	-0.03	-0.03	0.35[d]
	skinfold	-0.29[a]	-0.12	-0.02	-0.24	0.01

a: $p<0.05$, b: $p<0.01$, c: $p<0.005$, d: $p<0.001$

When measurements at each age were combined, thus presenting a wider range of values of all variables, there was a highly significant correlation between height and weight and all four bone variables. In boys, there were similar correlations between height and BMC, BW and aBMD, but a weaker correlation for vBMD. Weight was equally related to BMC and BW but more weakly related to aBMD and vBMD. In girls, height was equally related to BMC and BW, but less so to aBMD and vBMD. Weight was more strongly related to BMC than to the other bone variables, and the weakest correlation was to vBMD. Generally correlations were similar in boys and girls except for BW where they were stronger in boys.

In boys there were no significant correlations between total skinfolds and any bone variable at any age. In girls, skinfold thickness was positively related to BW at each age except 11 years and negatively related to aBMD and vBMD at 11 years. When the individual skinfolds of biceps and triceps were considered separately, similar results were obtained. In the pooled data from all ages, skinfold thickness was positively related to BMC, BW and aBMD (but not vBMD) in girls ($p<0.005$, 0.001, 0.05 respectively), but not in boys.

4.3.2 Rate of change in bone status

Changes in height in boys and girls in each 2 year interval and the six year interval are shown in Table 4.3.3 as mean, sd and range. In boys, there was a moderate increase in height from 11 to 13 years, a greater increase from 13 to 15 years and a considerably reduced increase from 15 to 17 years. In girls, the greatest increase in height occurred from 11 to 13 years followed by a much smaller increase from 13 to 15 years and almost no increase from 15 to 17 years. If boys and girls are compared with respect to pubertal development, changes in height in early, and mid to late pubertal years are similar between the sexes - early: girls 12.3 cm, boys 14.4 cm; mid-puberty: girls 5.9cm, boys 5.9cm.

Table 4.3.3

Changes in height (cm) in each of the 2 year intervals and the six-year interval in boys and girls, as mean, sd and range.

Age interval	11-13	13-15	15-17	11-17
Boys				
n	54	54	51	51
mean : sd	12.7 : 3.4	14.4 : 3.9	5.9 : 4.1	31.7 : 3.8
range	7.3-20.6	5.2-21.5	1-15.8	23.5-40.0
Girls				
n	48	47	42	42
mean	12.3 : 3.0	5.9 : 4.1	1.2 : 0.9	18.6 : 5.2
range	4.8-18.0	0-16.3	0-3.4	7.2-27.6

Table 4.3.4 shows the univariate correlation coefficients for the relation between changes in height and changes in each of the four bone variables at each of the two year intervals and the six year interval. In boys between 11 and 13 years, there was a highly significant positive correlation between change in height and change in BMC, BW, and aBMD, but not for vBMD. In girls there was only a significant correlation between change in BW and change in height. For the interval 13 to 15 years, in boys only, the change in BW was positively related to the change in height ($p<0.001$), and change in vBMD was weakly negatively related ($p<0.05$). In girls there was a significant positive correlation between change in height and change in all bone variables except vBMD.

In boys between 15 and 17 years, changes in BMC and BW were positively related to change in height but changes in density variables were not. In contrast in girls, changes in BMC, aBMD and vBMD were positively related to change in height, but BW was not. In the 6 year period 11 to 17 years, results were again different between the sexes. In boys there was a negative correlation between change in height and changes in BMC, aBMD and vBMD and a positive correlation between change in height and change in BW. In girls there was no correlation between change in height and changes in aBMD and vBMD, but a positive

correlation between change in height and changes in BMC and BW.

Table 4.3.4

Univariate correlation coefficients between changes in height and changes in each bone variable in each 2 year period and the 6 year period.

Age interval	n	ΔBMC	ΔBW	ΔaBMD	ΔvBMD
Boys					
11 - 13	54	0.77[d]	0.79[d]	0.38[c]	0.01
13 - 15	54	-0.03	0.56[d]	-0.18	-0.27[a]
15 - 17	51	0.41[d]	0.69[d]	0.06	-0.21
11 - 17	51	-0.38[c]	0.36[c]	-0.51[d]	-0.56[d]
Girls					
11 - 13	48	0.06	0.48[c]	-0.17	-0.21
13 - 15	47	0.51[d]	0.35[b]	0.32[a]	0.12
15 - 17	42	0.61[d]	0.17	0.56[d]	0.40[c]
11 - 17	42	0.47[d]	0.72[d]	0.16	-0.21

a: $p<0.05$, b: $p<0.01$, c: $p<0.005$, d: $p<0.001$

4.4 Bone age

Bone age was measured at the time of the final bone measurement at a mean chronological age of 17.4 years. The mean (sd) and range in boys were 17.7 (1.08), 14.0 - 19.0 years, and in girls 17.2 (0.74), 15.0 - 18.0 years. Nine (19%) boys and 10 (25%) girls had skeletal ages of 19 and 18 years respectively, corresponding to the skeletal age at which epiphyseal-diaphyseal fusion of the ulna and radius is complete.

4.4.1 Absolute bone status

Both boys and girls were divided into three groups according to their bone age. For boys these groups approximated to tertiles but for girls the group numbers were uneven due to the frequency of a bone age of 17.0 years. Bone variables at 17 years were compared across groups using ANOVA. The results are shown in Table 4.4.1. There were significant differences in aBMD and vBMD between groups, in both boys and girls and in BMC in boys, with those subjects more skeletally mature having greater values. There was no difference in BW between groups in either boys or girls, or in BMC in girls.

It follows from these results that bone variables at 17 years expressed as a percentage of the same sex adult mean values will also be different according to bone age group i.e. those subjects more skeletally mature will have attained a BMC and vBMD nearer to the adult mean. For those boys and girls with a bone age of 19 and 18 years respectively (i.e. the skeletal age at which the epiphyseal-diaphyseal fusion in the radius and ulna is complete), the bone variables at 17 years expressed as a percentage of the mean adult value are shown in Table 4.4.2. Boys had attained 95% of the mean adult male BMC and 92% of adult male vBMD. Girls had attained 103% of the mean adult female BMC and 97% of the adult female vBMD.

Table 4.4.1

Bone variables at 17 years in boys and girls according to bone age, and F value and probability for ANOVA between groups.

a,b denote pairs of significantly different groups

Boys Bone age n	≤ 17.0 15	17.5 - 18.0 19	18.5 - 19.0 14	F	p
BMC g/cm	1.26^{ab}	1.49^{a}	1.58^{b}	10.1	0.0002
BW cm	3.21	3.23	3.28	0.2	0.8
aBMD g/cm^2	0.39^{ab}	0.46^{a}	0.48^{b}	21.7	<0.0001
vBMD g/cm^3	0.39^{ab}	0.46^{a}	0.47^{b}	17.8	<0.0001
Girls Bone age n	≤16.5 7	17.0 19	17.5 - 18.0 14	F	p
BMC g/cm	1.07	1.12	1.20	2.9	0.07
BW cm	2.80	2.73	2.80	0.7	0.5
aBMD g/cm^2	0.38^{a}	0.41	0.43^{a}	6.1	0.005
vBMD g/cm^3	0.42^{ab}	0.46^{a}	0.47^{b}	5.1	0.01

Table 4.4.2

Bone variables at 17 years of skeletally mature subjects, expressed as a percentage of the mean same sex adult value.

	Boys n= 9		Girls n=10	
	BMC	vBMD	BMC	vBMD
Adult mean	1.707 g/cm	0.52 g/cm^3	1.175 g/cm	0.48 g/cm^3
Percentage adult mean	95.3	91.7	102.8	97.0

4.4.2 Rate of change in bone status

The change in bone variables between 15 and 17 years was compared across bone age groups using ANOVA. The results are shown in Table 4.4.3. In boys there were significant differences in BMC and BW across bone age groups with the change in these bone variables being greater in those subjects less skeletally mature. There were no differences in the changes in aBMD and vBMD according to skeletal age. In girls there were significant differences in BMC, aBMD and vBMD between bone age groups with more skeletally mature subjects displaying less change for each of these variables.

Table 4.4.3

Change (Δ) in each bone variable between 15 and 17 years according to bone age at 17 years. a,b denote pairs of significantly different groups.

Boys Bone age	≤ 17.0	17.5 - 18.0	18.5 - 19.0	F	p
n	15	19	13		
ΔBMC g/cm	0.30^{a}	0.28	0.23^{a}	3.4	0.04
ΔBW cm	0.30^{ab}	0.16^{a}	0.12^{b}	7.9	0.001
ΔaBMD g/cm^2	0.063	0.069	0.054	1.4	0.3
ΔvBMD g/cm^3	0.040	0.627	0.491	2.3	0.1
Girls Bone age	≤16.5	17.0	17.5 - 18.0	F	p
n	7	19	13		
ΔBMC g/cm	0.15^{ab}	0.09^{a}	0.07^{b}	5.6	0.007
ΔBW cm	0.08	0.04	0.07	0.8	0.5
ΔaBMD g/cm^2	0.045^{a}	0.026	0.016^{a}	5.4	0.01
ΔvBMD g/cm^3	0.047^{a}	0.03	0.016^{a}	3.2	0.05

DISCUSSION

4.1 Age and gender

4.1.1 Absolute bone status

Bone mineral (BMC) and bone size (BW)

The findings the present study of increasing BMC and BW with age are comparable with those of many studies which have used a variety of techniques and measured bone mass in both the axial, appendicular and total skeleton (Christiansen et al 1975a, Geusens et al 1991, Gunnes 1994, De Schepper et al 1991, Rico et al 1993c). Gender differences in bone mass and bone width have been reported by Geusens et al (1991) in the distal and proximal forearm. They found that in the five-year age group 10 to 15 years there were no sex differences in BMC or BW, but in 16 to 20 year-olds BMC of the proximal radius and BW of the proximal and distal radius were greater in males, findings similar to those of the present study. Christiansen (1975a) used single photon absorptiometry (SPA) of the forearm to estimate total body calcium and reported significantly greater values in girls than boys at 11 years, and in boys from 15 to 20 years, but with no differences between the sexes in the years 11 to 15. The greater bone mass in girls at 11 years is probably due to a cohort effect resulting in lower than expected values in the boys as total body calcium was lower in boys aged 10 and 11 years than those aged 9 years.

Measurements of the axial skeleton and total bone mass have shown that there are no sex differences in BMC in subjects less than 17 years of age but that males of 20 years and over have greater values than females (De Schepper et al 1991, Geusens et al 1991, Rico et al 1993c).

Differences in BMC with age and gender can be attributed to differences in stature as BMC is a function of bone size. Sex differences in height do not become apparent until 15 years of age.

Bone density (aBMD, vBMD)

The pattern of increasing aBMD with age in both sexes with a decline in the rate of increase in girls occurring around the age of 15 years (as found in the present study), has been reported from studies in the forearm by Landin and Nilsson (1981) and Rubin et al (1993) and from studies in the LS (Lu et al 1994). However gender comparisons have been more variable. Rubin et al (1993) found no sex differences in radial aBMD in 6 to 18-year-olds whilst Landin and Nilsson (1981) reported that boys aged 4 to 16 years had a significantly greater forearm aBMD than girls ($p<0.05$). Geusens et al (1991) in a study 3 to 25-year-olds reported that females aged 16 to 20 had greater aBMD at the distal radius than males, but 21 to 25-year-old males had greater aBMD of the distal and proximal radius than females. Gunnes (1994) in his study of 11 to 18-year-olds found aBMD was similar in boys and girls in the proximal (cortical) forearm, but 8.2% higher in boys in the ultradistal (trabecular) forearm. The results of the present study of no gender difference in aBMD except at 17 years when boys had greater value than girls most closely compare with those of Rubin and Gunnes

Measurement of aBMD at other sites has also produced variable results. Southard et al (1991) found no sex differences at any age in aBMD of the spine of 218, 1 to 19-year-olds whilst Kroger et al (1992) studying 84, 6 to 19-year-olds found that sex differences in aBMD of LS and femoral neck (FN) depended on the age of the subjects, with girls having greater mean values than boys at 12 to 13 and 16 to 17, but boys having greater values at 18 to 19 years. In this latter study numbers in each age group were very small. Faulkner et al (1993a) measured total body (TB) and regional aBMD in 234, 8 to 16-year-olds and found no sex differences in TB at any age, but significant overall gender differences in the upper limbs in which boys had greater values than girls, and the reverse in the pelvis in 15 to 16-year-olds. In a cross-sectional study in which aBMD of the TB, LS and FN was measured in 266 subjects aged 4 to 27 years, there were no sex differences in TB aBMD until the late teens when it became much greater in boys (Lu et al 1994). Girls had an earlier increase in age-related LS aBMD but there was no sex difference at peak aBMD. FN aBMD was no different in the mid-teens but greater in boys at peak aBMD. The results in the present study parallel these findings for TB and FN and indicate that changes in forearm aBMD, determined by SPA, reflect changes at other predominantly cortical sites.

These varying results in aBMD may reflect the heterogeneity in bone growth in adolescence. As this measure is not independent of bone size, variations in bone growth with respect to site,

gender and age will manifest as variations in aBMD. Estimation of BMD as a volumetric measure (vBMD) overcomes this problem.

A number of previous studies have measured or calculated vBMD. At some sites, vBMD increased with age (as in the present study), namely the forearm (Landin and Nilsson 1981, Katzman et al 1991), and LS (Katzman et al 1991, Kroger et al 1993, Gilsanz et al 1994), whilst at other sites FN (Katzman et al 1991, Kroger et al 1993), FS (Lu et al 1995) and TB (Katzman et al 1991) vBMD did not increase with age. These differences between sites may be due to the nature of the bone predominant at those sites. In the whole body, FN and FS where cortical bone is predominant an increase in bone mass will result from an increase in bone dimensions without a change in density. In the LS where trabecular bone is predominant, thickening of the trabeculae and addition of trabeculae may occur without a change in bone dimensions and these changes will result in an increase in bone density. This difference between bone density with age with respect to type of bone does not explain the increase in vBMD of the predominantly cortical forearm. Katzman et al (1991) proposed that an increase in forearm vBMD may result from endosteal bone apposition. As cortical bone is very dense a very small increase in cortical thickness will result in a significant increase in bone mineral.

With respect to sex differences Landin and Nilsson (1981) reported no sex differences in forearm vBMD in 4 to 16-year-olds and similarly Gilsanz et al (1994) found no sex differences in LS vBMD of 195, 4 to 20-year-olds in either cortical or trabecular bone. These findings are in agreement with the present study when the entire age range is considered although at some individual ages there were significant differences between sexes. Conversely Lu et al (1995) reported that FS vBMD of 204, 8 to 27-year-olds was higher in males than females ($p<0.05$) across the age range.

No previous study has reported a fall in forearm bone density in boys in the early pubescent years as found in the present study of vBMD. The cross-sectional design and the measurement of bone density as an areal measure in most studies, result in differences in bone size confounding differences in bone density and may result in failure to detect a fall in this measure.

These results illustrate the heterogeneity of bone growth in the second decade with respect to magnitude, site, gender and age. Without exception these studies report a rapid increase in

BMC, bone size and aBMD during adolescence. The observed sex differences may be explained by differences in bone size between boys and girls, and the differences between studies may be explained by the heterogeneity of bone growth and bone mineral accrual in this period. vBMD corrects for bone size and so removes one artifact from the comparisons between boys and girls.

4.1.2 Tracking

In this study there was a high degree of tracking in all four bone variables in both sexes. Tracking of vBMD was weakest but became established from 13 years in girls and 15 years in boys. Tracking was weakest in the six-year interval 11 to 17 years which in most subjects represents a transition from pre-pubertal to post-pubertal. The weaker tracking of BMD in 2-year intervals in mid-puberty in boys, reflects the wide scatter of levels of maturity at any given age resulting in a wide range of values in the bone variables. This wide scatter is confirmed by the higher coefficients of variation in individual bone variables in the mid-pubertal years. The consistently higher tracking coefficients of vBMD in girls than boys suggest that there was a smoother rate of change in this variable in girls. This may be a result of the shorter time frame within which the pubertal changes occur. No other studies have reported tracking.

The high degree of tracking in all bone variables was comparable with the degree of tracking of height and suggests that there is limited opportunity for environmental factors to modify peak bone mass and density. However the weaker tracking of vBMD indicates the possibility of greater modification of bone density than bone mass.

4.1.3 Rate of change in bone status

Bone mineral content (BMC) and bone width (BW).

Cross-sectional studies of the forearm and other regions have reported that increases in BMC in girls are much less after 15 years whereas increases continue in boys (Bonjour et al, 1991, Rubin et al 1993) a finding comparable with the present study. Similar results of increases continuing to a later age (up to 20 years) in boys than in girls have been reported from short-term longitudinal studies (Kroger et al 1993, Theintz et al 1992). In addition increases in boys were greater than in girls a finding also reported by Kroger et al (1993) in the FN but not in the LS.

Although the maximum increase in BW in girls occurred between age 11 and 13 in the present study it is likely that there was a significant increase in the year before the first measurement judging by the increase in height which occurs from age 10 years in girls (Tanner and Davies 1985). This is supported by the findings of Goslings et al (1995) of increased velocities of BMC and BW in girls from the age of 7.

Bone density (aBMD,vBMD)

Several cross-sectional studies have reported a decline in the rate of increase in aBMD of the forearm (Landin and Nilsson 1981, Rubin et al 1993) and FN, FS and LS (Bonjour et al 1991), after 15 years of age in girls, but a continued increase in boys, as found in the present study. Similar results were reported in two longitudinal studies of one-year (Theintz et al 1992, Kroger et al 1993) in which aBMD of the LS, FN and FS was measured. Both Kroger and Theintz reported that maximal rates of increase occurred between 13 and 17 years of age in boys, a similar finding to the present study. In contrast, the most marked increases in girls occurred between 11 and 13/14 years compared with 13 to 15 years in the present study. However annual percentage increases at the time of greatest change were similar in all studies. Variations in rates of change in aBMD are influenced by the rate of change in bone size so a more accurate picture of the magnitude of the change in bone density is obtained from vBMD.

In the present study a similar pattern of change in vBMD as in aBMD was seen with respect to timing of maximal change and gender differences and this has also been reported by Kroger et al (1993) in their one-year longitudinal study. However they found that the annual increase in vBMD at the time of maximal change was only half the increase in aBMD, compared with about 70% in boys and no difference in girls in the present study.

Differences between studies may arise from differences in the site of measurement. Increases in aBMD may reflect increased bone size and/or a true increase in density. If increased bone size is the predominant factor the increase in vBMD will not be as great as that in aBMD. Increases in vBMD may result from increased cortical thickness through endosteal bone apposition (as in the forearm) or increased thickness and number of trabeculae as in LS. Due to the high density of cortical bone, the former will have a greater effect on vBMD than the latter and hence result in differences between sites.

The results of the present study indicate more clearly the true pattern of bone mass accumulation over the adolescent period as it is longitudinal and so eliminates inter-subject variation. It shows that there is considerable bone growth and consolidation during adolescence with cessation of growth occurring before BMC and BMD. Although BMC and BMD continue to increase to age 17, the rate of increase slows markedly in females after age 15, while remaining high in males to age 17. For how long BMC and BMD continue to increase cannot be determined from this study, but the high rate of increase still occurring in boys from 15 to 17 years suggests that bone mass may continue to increase for some years. Other longitudinal studies indicate that gains over one year are not statistically significant in girls after about 17 years of age although gains measured over a longer period may be significant. These studies suggest that most bone has been acquired by 17 years of age in girls and 20 years of age in boys.

The net loss of vBMD in boys between 11 and 13 years has not been described previously. This fall in vBMD may be explained by an increase in porosity of cortical bone brought about by a large increase in bone turnover. This increase in bone turnover at the time of rapid longitudinal growth provides some of the mineral required for the new bone formed at epiphyseal growth plates (Parfitt 1981). As longitudinal growth is not as great in girls as in boys, there is not as great an increase in bone turnover, and hence less of a change in porosity (Krabbe et al 1980). Had measurements been taken at 10 years, a fall in vBMD may have been observed in girls from 10 to 11 years, at the time of the pre-pubertal growth spurt. This fall in vBMD in early puberty may contribute to the increased fracture rate that occurs at this time in both boys and girls (Ogden 1982, Mizuta et al 1987).

4.1.4 Peak bone status

PBM is the greatest value attained before age- and menopause-related bone loss begins. Although PBM strictly refers to bone mass, the term is used loosely in the literature to refer to both BMC and BMD though usually the latter. Age at attainment of PBM varies with the region of the skeleton (Gordon and Webber 1993) and the type of bone (Jelic et al 1992). Studies of the predominantly cortical bone of the forearm suggest that aBMD at this site remains unchanged in the third decade (Sowers et al 1985, Rico et al 1993b) or increases very

slowly (Recker et al 1992, Rico et al 1992). No studies have previously reported values in forearm vBMD during growth. Although it is not possible to determine the age at attainment of PBM in the forearm from the present study, the rapid increase in vBMD in boys between 15 and 17 years suggests that it will continue to increase in subsequent years. The slower increase in vBMD in girls between 15 and 17 years does not rule out further increases but suggests that any increases beyond 17 years will be much smaller than those expected in boys.

Comparison of adolescent values with adult values has also been used as a means of determining timing of PBM. However, there are no reports comparing any measure of bone density (aBMD or vBMD) of boys with adult values, or comparing vBMD of the forearm in girls, with adult values. In the present study BMC and vBMD in 11 year-old boys and girls represented about half and just over two-thirds of the same sex adult mean BMC and vBMD respectively, increasing to within 15% of BMC and vBMD in boys and 5% in girls by age 17. Most studies comparing adolescent values with those of adults have used aBMD which does not entirely account for possible differences in bone size between the groups; for example 14 to 15-year-old girls had an aBMD of LS, FN and FS of 99%, 105% and 94% respectively of 20 to 35-year-old women (Bonjour et al 1991). Girls aged 16 had attained adult pre-menopausal levels of forearm aBMD, with levels in the ultradistal site being attained earlier than those in the distal forearm (Gunnes 1994). However as the aBMD of the distal forearm was still increasing from 15 to 16 years it is probable that further increases would occur.

Comparison of vBMD will more accurately reflect differences in bone density. Gilsanz et al (1988a) compared a group of 14 to 19-year-olds and 25 to 35-year-olds and found the former had greater trabecular vBMD in LS and from this observation suggested that PBM was attained in females in the late teens, around the time of cessation of growth. These findings from both aBMD and vBMD measurements suggest that PBM may be attained earlier in predominantly trabecular bone than in predominantly cortical bone. In the present study the significantly lower vBMD values of 86% and 94% of the same-sex mean adult values in boys and girls respectively, suggest that forearm vBMD has not reached a peak value by age 17.

4.2 Pubertal status, age of menarche, sex hormones

4.2.1 Absolute bone status

Pubertal status

In the present study increases in BMC and BW were related to maturity as determined by PHS. At each stage boys had greater values than girls. These findings are similar to those from the cross-sectional studies of Katzman et al (1991) and Bonjour et al (1991). Rico et al (1993c) reported greater TB BMC in boys and girls at stages 4 and 5 than at stages 1 and 2. There were no gender differences at stages 1 and 2, unlike the present study, but boys had greater values than girls at stages 4 and 5 as reported in the present study. In a longitudinal study of 35 boys, initial age 11 to 12 years, followed for two and a half years, Krabbe and Christiansen (1984) reported that forearm BMC progressively and significantly increased from three months before PHS 2 to PHS 4. The increase from stage 2 to stage 4 was 20%. In the present study there was a much greater increase of 50% from PHS 2 to PHS 4, however it is possible that many of the boys may have been at this latter stage for some time when the second bone measurement was made.

The finding that aBMD also increased with increasing sexual maturity has been reported in cross-sectional studies of forearm, LS, FN, and TB (Rubin et al 1993, Grimston et al 1992, Katzman et al 1991, Bonjour et al 1991). With respect to gender differences Bonjour et al (1991) reported that aBMD of FN and FS (but not LS), at any given stage, was greater in boys than in girls, whereas Rubin et al (1993) reported that aBMD of LS was significantly greater in boys than girls at stages 1 and 3. The results of the present study fall between these two with aBMD being greater in boys than girls at stages 1, 2 and 5 but no different at stages 3 and 4. Gilsanz et al (1988b) reported a highly significant effect of puberty on spinal trabecular and cortical vBMD but no significant effect of gender or interaction between gender and pubertal status, a similar finding to the present study. In addition, Mora et al (1994), reported that cortical vBMD increased with each stage of puberty whereas trabecular vBMD was only greater at the later stages (4 and 5), again demonstrating substantial differences between cortical and trabecular bone in the pubertal pattern of growth.

Rubin et al (1993) proposed that there were two parallel but distinct aspects of skeletal growth during puberty, namely accelerated linear growth and accelerated bone accrual mediated by hormones and growth factors. They suggested that the hormonal effects of puberty were

stronger in determining axial aBMD of LS (predominantly trabecular bone) in comparison with peripheral aBMD of the radius (predominantly cortical bone). The onset of the age-related accelerated phases of axial aBMD accrual corresponded to the time of onset of the pubertal growth spurt in both sexes (early puberty in girls and mid-puberty in boys). In contrast, radial aBMD displayed steady growth related increases throughout most of childhood and adolescence.

Because changes in pubertal stage are closely associated with age, it is difficult to distinguish the two with respect to bone growth. In the present study bone variables increased with PS but within any particular pubertal stage there was a tendency to greater values in the older subjects. A three-way analysis of variance of age, PS and gender with each bone variable, indicated that all three variables had a significant effect on BMC and BW, age and PS but not gender had a significant effect on aBMD, and only PS and gender were significantly related to vBMD.

Gordon et al (1991) reported that aBMD of LS was marginally greater in females compared with males. The only increase in aBMD after age 10 in females was associated with puberty, whereas aBMD in males increased steadily throughout the age range 3 to 30 years, and the increases in aBMD associated with puberty were smaller in males than females. They proposed that because pre-pubertal growth continues longer in boys than in girls it contributes more to bone mass. They showed that growth during puberty contributes little (15%) to peak bone mass in males, but that approximately half the PBM accumulates at the time of puberty in females, and that bone mass does not increase after the early teenage years. Gilsanz et al (1988) also showed that vBMD of LS did not change with age, after accounting for puberty, and they also found there was no significant gender effect. This latter difference between Gilsanz and the present study may reflect differences between the sites under study.

Age at menarche.

The median age at menarche (12.9 years) of the present sample was slightly greater than that of a previous Australian study (12.6 years) (Penfold et al 1980). Few studies have reported an association between time of menarche and bone status. By measuring bone variables over a six-year period and thus encompassing pre- and post-menarchic years, the present study allows a more precise determination of the relationship between all bone variables and time of

menarche. While there was no relationship between time of menarche and any bone variable when subjects were more than two years post-menarche, within two years BMC, aBMD and vBMD were greater in those furthest post-menarche, a finding also reported by Lu et al (1994) in aBMD of TB, FN and LS. In contrast Turner et al (1992) in a study of 15 to 17-year-olds, reported a very weak trend to a lower aBMD of LS in those with menarche after 15 years. However those with late menarche (after 15 years) would have been within 2 years of menarche and still within the period of rapid increase. Similarly a study of vertebral aBMD in 63 premenopausal women aged 19 to 40 years found that women in the lowest quartile for aBMD had a significantly later age at menarche than those in the upper three quartiles (13.9 v 12.8 years $p<0.02$) (Armamento-Villareal et al 1992). Age at puberty as a determinant of PBM has also been demonstrated in men. In a study of 44 men aged 23 to 29 years, Finkelstein et al (1992) reported that the 23 subjects with a history of delayed onset of puberty (after 15 years), but a subsequent normal sexual maturation, had significantly lower radial and spinal aBMD than the 21 normal subjects.

Sex hormones

The weak association of oestradiol levels with bone variables may result from the wide normal range in oestradiol levels and the absence of information on the time of the cycle when samples were taken. Other studies in adolescents have not used oestradiol levels alone as a measure of developmental status, but as one of a number of factors to determine an oestrogen exposure score (Dhuper et al 1990, Lloyd et al 1992, Armamento-Villareal 1992). The other factors were onset of menarche, Tanner stage, number of menstrual cycles per year and oestradiol excretion. Dhuper et al (1990) however did report that in girls aged 13 to 20 years, there was no correlation between oestradiol levels and bone density. The association between bone mass and time of menarche in the present study suggests that in adolescents, time from menarche is a better indicator of stage of pubertal maturing than is serum oestradiol.

The strong positive correlation between testosterone and bone variables in this study is comparable with that of Krabbe et al (1979). From a cross-sectional study of 103 boys aged 7 to 20 years, they reported that increases in testosterone occurred simultaneously with increases in skeletal growth and forearm BMC. In the present study, the correlation between testosterone levels and bone density at 15 years (but not at 13 years), reflects the time lag in increases in aBMD and vBMD following increase in bone size. However as bone mass accrual

was incomplete in most of the boys in the present study, it is not possible to determine if the timing of puberty in boys influences PBM as suggested by Finkelstein et al (1992).

4.2.2 Rate of change in bone status

Pubertal status

In the present study rates of change in bone variables were related to stage of puberty. Maximum rate of change in BW occurred before puberty whereas maximum consolidation of bone occurred later in both sexes although boys did not display such marked differences between stages as girls. This recalls the gender differences in response to puberty suggested by Gordon (1991) and the observation of Kroger et al (1993) that pubertal development in boys is slower than in girls. No other studies have related rate and extent of change in bone growth to stage of puberty.

Age at menarche

Bonjour et al (1991), from a cross-sectional study, reported a substantial increase in aBMD of LS, FN, FS, and BMC and surface area of LS, in the first two years post-menarche, a moderate increase in the 2 to 4 years after menarche, but after 4 years a decrease in aBMD. Similarly Gunnes (1994), who measured aBMD of the distal and ultradistal forearm, reported increases to be greatest in the first two years after menarche, and that aBMD of the distal radius, (but not the ultradistal radius), continued to increase significantly in the third year post-menarche. Results from longitudinal studies are similar. Theintz et al (1992) in their one-year longitudinal study in 98 females aged 9 to 19 years, observed significant increases in aBMD of LS, FN and FS during the first 2 years post-menarche, but the mean increases were not significant from the second to the fourth year after menarche and beyond. Kroger et al (1993) reported that maximum increase in vBMD occurred at the time of menarche. The relations of rate of change in bone variables to time from menarche in the present study are comparable to those from cross-sectional and shorter longitudinal studies, but with additional data here on bone size, which changed maximally in the pre-menarchic period.

Sex hormones

The meaning of the association between change in BMC, aBMD and vBMD from 13 to 15 years and the oestradiol level at 15 years is unclear. Studies which have determined oestradiol levels as part of an oestrogen exposure score, have not reported changes in bone mass. Given the fluctuation in oestradiol levels within a menstrual cycle, time of menarche is probably a better indicator of hormonal status in the adolescent years.

A longitudinal study of 18 boys by Riis et al (1985), in which forearm BMC and testosterone levels were measured every three months, reported that markers of bone formation (alkaline phosphatase and Gla protein) increased concomitantly with serum testosterone whereas the maximal increase in bone mass followed some 10 months later than the maximal increase in testosterone. In the present study there was no association between testosterone levels at the initial age or increase in testosterone in the two year period, and the increase in bone variables for either 11 to 13 years or 13 to 15 years. As measurements were made at two year intervals it was not possible to identify the time of maximal increase in testosterone.

4.3 Anthropometric measures

4.3.1 Absolute bone status

The narrow range of height, weight and skinfold thickness at each age may account for the lack of correlations between these measures and bone variables in the present study. When the data from all ages was pooled the results are similar to many reports of the positive associations between bone variables measured at a range of sites and height and weight, both with respect to the strength of the correlations and the tendency to weaker correlations with vBMD than BMC (Katzman et al 1991, Lloyd et al 1992, Kroger et al 1993, Mora et al 1994). The lack of significant correlations between total skinfold thickness and all bone variables in boys compared with positive correlations with BMC, BW and aBMD in girls may reflect the increase in skinfold thickness which accompanied ageing in girls but not in boys. There are few reports of the relation between body fat and bone mass in children. Miller et al (1991) reported that fatness measures were seldom significant predictors of bone density in children and that height was the single best predictor of bone mass at all bone sites in both sexes. Rico et al (1994) reported that in 14 to 18-year-old girls, TB aBMD was correlated with lean body

mass but not with body fatness. These results contrast with reports in adults and suggest that obesity is not an associated variable when other measures of body size are taken into account.

4.3.2 Rate of change in bone status

In the present study the different correlations between rate of change in height and rate of change in bone variables according to gender and time interval reflect gender differences in the timing and magnitude of skeletal growth. Rate of change in BW reflects skeletal growth and hence is strongly correlated with rate of change in height at the peak of the growth phase (girls 11-13 years, boys 11-15 years). Although changes in BMC also reflect changes in bone size, the lack of an association between rate of change in BMC and change in height in the mid to late growth phase (girls 11-13, boys 13-15) may be because bone size had increased so much in the previous four years that density had actually decreased ie BMC had not kept up with the increase in BW. Kleerekoper et al (1981) discussed this phenomenon with reference to the work of Krabbe et al (1980), and suggested that BMC increased only moderately when the most rapid increase in height was occurring. They attributed this to increased porosity in cortical bone due to increased bone turnover which provided some of the mineral for the newly formed bone at the epiphyses. Such an increase in porosity is demonstrated in the present study by a fall in vBMD when bone growth is rapid. Kleerekoper et al (1981) proposed that when longitudinal growth slowed, BMC increased more rapidly, and hence changes in height were followed by changes in BMC. This occurs later in boys than in girls (boys: 15-17 years, girls:13-15 years).

A similar explanation may apply to the positive correlation in girls and negastive correlation in boys between change in height and change in BMC across the six-year interval 11 to 17 years. This period includes four years of rapid growth in boys but only two years of rapid growth in girls, followed by four years of much slower growth.

The negative correlations in boys between change in height and change in aBMD and vBMD follow from this explanation. While these density variables increased in this six-year interval, the overall change in BMD was smaller in those subjects who grew the most, because of an initial fall in BMD due to the rapid growth in the early years. Conversely a relatively shorter period of growth and one of less magnitude in girls did not result in a fall in BMD and hence

there was no association between change in height and change in BMD.

From their longitudinal study Kroger et al (1993) reported that annual height gain was significantly related to annual increases in BMC, BW and aBMD (but not vBMD) of LS and FN. As their study included subjects over a much wider age range than the present one, the negative associations observed at the time of rapid growth in the present study may have been masked by positive associations at times of slower growth. Theintz et al (1992) also reported a statistically significant positive relation between changes in height and changes in BMC of LS in 9 to 19-year-olds. However when subjects were grouped according to PS at initial measurement, the mean increase in bone mass for the same gain in statural height varied with PS, in both females and males.

4.4 Bone age

4.4.1 Absolute bone status

Previous studies relating bone status to bone age have been based on younger subjects than those in the present study but have similarly found bone status to be related to bone age (Glastre et al 1990, Trouerbach et al 1991). Mazess and Cameron (1971) also reported that bone age was significantly correlated with radial BMC, BW and aBMD, but that height and weight were better predictors of BMC than skeletal age. The gender differences in the relation between bone age and each of the bone variables at 17 years in the present study can be explained by the wider range of bone age values at 17 years and the fact that significant consolidation continues to a later age in boys.

The observation in the present study that the skeletally mature girls had attained mean BMC and vBMD similar to mean adult female values suggests that these girls were near peak bone mass. As bone age was measured only once, the precise chronological age at which skeletal maturity was reached in these subjects is not known. The relatively lower bone values in the skeletally mature boys, suggest that the boys are further from PBM than the girls. As vBMD was less than 100% of the young adult mean in both boys and girls, it is likely that peak vBMD has not been reached at the time of cessation of longitudinal growth, ie bone consolidation continues beyond this point.

4.4.2 Rate of change in bone status

Gender differences in the relation between rate of change of bone variables and bone age result from the fact that at age 17 there was still considerable increase in all bone variables, except BW, in most boys but in many girls, bone variables were past peak velocity and so there was a wider range of values. These findings indicate that increases in bone density are smaller in girls near skeletal maturity than in those less mature, whereas increases in bone density in boys are as great in the skeletally mature subjects as in those less mature. These findings have implications for the timing of PBM with respect to skeletal maturity. They suggest that bone consolidation continues well beyond skeletal maturity in boys but that there is little bone consolidation beyond this point in girls. This gender difference is probably due to the protracted response to testosterone in boys. Muscle mass, and hence force acting on the bone, increases beyond skeletal maturity well into the third decade.

4.5 Summary

The results of the present study show that measures of forearm bone mass, size and density, namely bone mineral content, bone width, areal bone mineral density and volumetric bone mineral density, all increase with age and sexual maturity. Absolute values of BMC, BW and vBMD (but not aBMD) were dependent on gender when age and sexual maturity were controlled for. Velocity of each bone variable was dependent on age and sexual maturity, and the response to these factors differed between variables. In contrast with the other bone variables vBMD velocity from 11 to 13 years was negative in boys and zero in girls. Maximal increases in all bone variables occurred chronologically earlier in girls, and earlier in BW than in BMC, aBMD and vBMD in both sexes. These gender differences could be attributed to the later sexual maturity in boys. BW velocity decreased with increasing sexual maturity, but BMC, aBMD and vBMD velocities increased.

BMC and BW were greater in boys than girls at the older ages, but there were few gender differences in aBMD and vBMD. In the six year period 11 to 17 years, the percentage increases in BMC and BW were greater in boys than girls. There was no gender difference in the increase in aBMD, but vBMD increased more in girls than in boys. The measure of BMD

as vBMD accounts for age and gender differences in bone size, hence differences in vBMD are true differences in mineral density. There was a high degree of tracking in all bone variables and the tracking coefficients were comparable to those of height.

Rates of increase in bone variables from 15 to 17 years of age, and comparison of values at age 17 with adult values, suggest that girls are near PBM by the age of 17, but in boys significant gains are still occurring.

Bone variables increased with increasing skeletal maturity as determined at age 17. This relationship was significant in aBMD and vBMD in both boys and girls, and in BMC in boys. In the skeletally mature subjects, the relation of bone variables to adult values, and the very low rate of change in the previous two years suggest that girls are very close to PBM at this stage, but that boys are up to 10% below PBM. Thus bone consolidation continues after cessation of longitudinal growth in boys, but while this may also occur in girls, it is much less.

The results support the hypothesis that peak bone mass is reached near skeletal maturity in girls but they do not support this hypothesis in boys.

CHAPTER 5

RESULTS: BONE STATUS AND ENVIRONMENTAL FACTORS

5.1 Nutrient intake

5.2 Physical activity

5.1 NUTRIENT INTAKE

5.1.1 Intake of energy and nutrients

The number of subjects by age and sex for whom nutrient intake data is available is shown in Table 5.1.1. The daily calcium intakes are shown in Table 5.1.2 expressed as the mean, sd, range and 10th, 50th and 90th percentiles. Calcium intake increased with age in boys from 4 to 17 years. In girls intake increased up to age 11 and then remained almost unchanged from 11 to 17 years, although intake at 15 years was lower than at the other teenage years. Intakes were consistently greater in boys than in girls and these differences were significant at all ages from 8 years (8y $p<0.01$, 11-17 y $p<0.001$). In both boys and girls there was a wide range of intakes at every age .

Table 5.1.1
The number of subjects by age and gender for whom nutrient intake data is available

Age years	4	6	8	11	13	15	17
boys	57	57	56	54	52	52	44
girls	43	42	44	52	49	47	38

Table 5.1.2
Mean, standard deviation (sd), range and 10, 50 and 90th percentiles of calcium intakes by age and gender (mg/day).

Age years	Mean	SD	Range	Percentiles 20	50	80
Boys						
4	736	221	321-1301	553	707	881
6	786	240	373-1497	591	749	936
8	882	314	360-1707	628	826	1120
11	1090	390	384-2122	709	1083	1360
13	1126	426	399-2410	801	1071	1420
15	1240	531	523-2514	770	1087	1722
17	1364	703	249-3522	830	1295	2163
Girls						
4	687	211	241-1161	497	655	979
6	724	215	432-1155	493	730	877
8	728	247	217-1539	580	712	916
11	823	248	287-1366	605	809	1004
13	829	341	323-2037	526	764	1002
15	737	301	236-1423	450	692	950
17	809	405	314-2418	534	710	974

Mean daily intakes of energy, protein, sodium, phosphorus, fibre, and vitamin D are shown in Table 5.1.3, expressed as the means and sd. Intakes of energy and nutrients showed similar trends to those observed for calcium intake, namely intakes in boys increased at every age while those in girls increased up to 11 years and then generally remained static or declined a little in the following six years. Similarly boys had higher intakes than girls at every age. The gender differences were not significant at age 4 or 6 years (except for energy intake ($p<0.05$)), but were significantly greater in boys at age 8 for all nutrients ($p<0.01$) and all but vitamin D intake at ages 11 to 17 years ($p<0.001$ for all).

When expressed as nutrient densities (g/mg per MJ energy), there were few age or gender differences in nutrient intakes, indicating that the higher intakes in boys and older subjects were principally a result of the greater overall food intake. This greater food intake can be

attributed to an increase in body size, although when expressed as energy intake per kilogram body mass, intake was greatest at age 4(boys: 325, girls: 309 KJ/kg) and declined in both sexes to its lowest value at age 17 (boys: 153, girls:112 KJ/kg). Gender differences were apparent from the age of 6 years and were related to differences in muscle mass and possibly activity level. Table 5.1.4 shows the mean nutrient densities of protein, calcium, sodium, fibre, vitamin D and phosphorus. Boys had significantly greater protein and phosphorus densities at 11 years ($p<0.01$) and protein density was greater in 11 to 17-year-old boys and 13 to 17-year-old girls than in those aged 4 to 8 years.

Calcium balance is affected by a number of nutrients so the ratio of calcium to these nutrients in the diet is relevant. The mean calcium/protein, calcium/sodium, calcium/fibre and calcium/phosphorus ratios are shown in Table 5.1.4. There were few gender differences. Boys had a significantly greater calcium/sodium ratio at 13 and 15 years, and calcium/fibre ratio at 11 years ($p<0.05$). The calcium/protein ratio decreased in both boys and girls from age 4 to 17 years and was significantly greater at ages 4 and 6 than at ages 13 and 15 years ($p<0.001$).

The percentage of subjects with intakes below the recommended dietary intake (RDI) and 0.7 RDI at each age and in each sex are shown in Table 5.1.5 together with the relevant RDI. 0.7 RDI is the conventional cut-off point used by dietitians in clinical practice when assessing the adequacy of an individual's diet. A large proportion of both boys and girls had intakes below the RDI at every age. In boys the range was from a low of 20% at age 11 to a peak of 67 % at four years, and in girls from 67% at ages 6 and 8 to 85% at 15 years. At each age fewer boys than girls had a calcium intake below 0.7 RDI. Between a quarter and a third of girls had a calcium intake <0.7 RDI at each age, but over half fell below this point at age 15.

Table 5.1.3
Daily intakes (mean, sd) of energy (MJ), protein (g), sodium (Na mg), fibre (g), vitamin D (Vit D mg), phosphorous (P mg) in boys and girls from ages 4 to 17 years.

Age years	n	Energy		Protein		Na		Fibre		Vit D		P	
Boys													
4	57	5.88	0.96	50	11	1718	508	12.4	4.7				
6	57	6.43	0.97	53	12	1857	514	13.3	5.1				
8	57	7.36	1.23	63	13	2182	574	15.9	5.6				
11	54	8.83	1.52	79	16	2457	625	17.7	6.5	2.8	3.0	1403	304
13	52	9.07	1.73	85	18	2683	769	19.1	7.3	2.6	2.3		
15	52	10.19	2.15	97	26	2970	903	20.9	7.3	3.3	4.6		
17	44	10.88	2.31	107	28	3227	1030	21.9	9.5	3.01	1.7	1785	548
Girls													
4	43	5.55	1.09	46	11	1644	396	11.4	4.4				
6	42	5.98	1.07	51	12	1875	588	12.0	3.8				
8	44	6.59	1.38	57	12	1879	464	12.8	3.8				
11	52	7.57	1.63	63	14	2139	511	15.4	4.9	2.6	2.3	1086	245
13	49	7.46	1.46	67	17	2313	586	14.9	4.4	2.6	2.4		
15	47	6.95	1.61	63	15	2119	635	14.5	7.0	2.2	1.7		
17	38	6.92	1.73	66	18	2155	634	15.5	6.2	2.7	3.0	1096	350

Table 5.1.4
Nutrient densities and nutrient density ratios of the mean daily intakes of protein (g/MJ), calcium (Ca mg/MJ), sodium (Na mg/MJ), fibre (g/MJ), vitamin D (Vit D)(mg/MJ), phosphorous (P)(mg/MJ), and the Ca/Protein (Ca/pro), Ca/Na, Ca/fibre and Ca/P ratios.

Age years	Protein	Ca	Na	Fibre	Vit D	P	Ca/pro	Ca/Na	Ca/fibre	Ca/P
Boys										
4	8.4	125	292	2.14			15.0	4.5	69	
6	8.3	122	289	2.10			14.8	4.4	71	
8	8.6	119	297	2.18			14.0	4.2	61	
11	9.1	125	277	2.03	0.31	160	13.8	4.6	71	0.76
13	9.5	124	298	2.11	0.30		13.1	4.4	65	
15	9.6	122	291	2.08	0.31		12.9	4.3	68	
17	10.0	123	295	2.02	0.27	163	12.5	4.3	67	0.74
Girls										
4	8.4	125	300	2.09			15.0	4.4	68	
6	8.5	122	312	2.02			14.7	4.0	68	
8	8.7	111	289	1.94			12.9	4.0	60	
11	8.4	111	287	2.06	0.36	145	13.3	4.0	57	0.75
13	9.1	106	306	2.08	0.33		12.3	3.7	60	
15	9.1	106	306	2.08	0.33		11.6	3.7	57	
17	9.7	116	319	2.26	0.38	159	12.1	3.9	55	0.72

Table 5.1.5
The percentage of subjects with a mean calcium intake below the RDI and below 0.7 RDI, and the
RDI (mg calcium) by age and gender.

Age years	4	6	8	11	13	15	17
Boys							
RDI	800	800	800	800	1200	1200	1000
%<RDI	67	56	46	20	65	58	32
%<0.7 RDI	21	16	9	9	25	29	16
Girls							
RDI	800	800	900	900	1000	1000	800
%<RDI	72	67	77	67	76	85	68
%<0.7 RDI	30	29	23	29	39	53	29

5.1.2 Tracking of nutrients.

Table 5.1.6 shows the tracking coefficients of mean daily energy, absolute calcium intake and calcium density between each age in boys and girls separately. The degree of tracking in all three variables decreased as the time interval increased. In boys, tracking of absolute calcium intake was greater than energy intake, but varied with age in relation to the tracking of calcium density. In girls in the prepubertal years, tracking of energy was slightly greater than either calcium intake or calcium density. In the pubertal years, tracking of energy and calcium were similar but less than calcium density. The degree of tracking of calcium density was similar in boys and girls in the adolescent years (11 years and beyond), but generally greater in boys in the pre-pubertal years.

Table 5.1.6

Matrix of tracking coefficients between ages 4 to 17 years of mean daily food energy (top line), absolute calcium intake (mid line) and calcium density (bottom line). Boys to right and girls to left of diagonal.

Boys: $r>0.30$ $p<0.01$, $r>0.35$ $p<0.005$, $r>0.40$ $p<0.001$. Girls: $r>0.33$ $p<0.01$, $r>0.38$ $p<0.005$, $r>0.46$ $p<0.001$.

	4	n	6	n	8	n	11	n	13	n	15	n	17	n
4			0.38	57	0.28	56	0.17	54	0.34	52	0.38	52	0.17	44
			0.46		0.33		0.13		0.22		0.11		-0.01	
			0.58		0.58		0.19		0.25		0.22		-0.04	
6	0.63	41			0.51	56	0.31	54	0.41	52	0.44	52	0.36	44
	0.47				0.68		0.55		0.40		0.41		0.28	
	0.27				0.62		0.41		0.31		0.46		0.16	
8	0.54	43	0.34	42			0.42	54	0.40	52	0.44	52	0.30	43
	0.51		0.37				0.71		0.55		0.47		0.49	
	0.43		0.16				0.69		0.47		0.49		0.44	
11	0.35	42	0.27	41	0.54	43			0.26	50	0.22	50	-0.05	41
	0.28		0.27		0.43				0.52		0.56		0.46	
	0.39		0.13		0.33				0.44		0.49		0.52	
13	0.46	40	0.32	39	0.29	41	0.40	48			0.44	51	0.29	41
	0.40		0.25		0.35		0.40				0.58		0.63	
	0.45		-0.06		0.34		0.44				0.62		0.55	
15	0.23	39	0.42	38	0.18	40	0.28	46	0.34	47			0.46	41
	0.16		0.15		0.36		0.36		0.34				0.62	
	0.37		0.07		0.39		0.49		0.62				0.62	
17	0.12	32	0.04	31	0.11	33	0.20	37	0.34	37	0.27	36		
	0.41		0.25		0.12		0.02		0.28		0.30			
	0.28		0.13		0.38		0.52		0.55		0.55			

5.1.3 Calcium intake over time

A measure of habitual calcium intake was determined in three epochs by calculating the mean calcium intake at the 4, 4, and 7 occasions of measurement in each age range respectively ie Epoch 1: 4-11 years, Epoch 2: 11-17 years, and Epoch 3: 4-17 years. Table 5.1.7 shows the mean, sd and range of calcium intake of each of these periods. The number of times a subject had a calcium intake below the RDI and 0.7 RDI in each of these three time periods was also determined. The results are shown in Table 5.1.8. More girls than boys had intakes consistently below the RDI in each epoch, and more boys than girls had intakes consistently above the RDI. In epoch 3 (7 occasions of measurement) three boys but no girls had intakes above the RDI at every occasion, and one girl but no boys had intakes below 0.7 RDI at every occasion.

Table 5.1.7
Calcium intakes (mean, sd, and range) in mg/day in the three epochs 4 to 11 years, 11 to 17 years and 4 to 17 years by gender.

Epoch	1: 4-11 years	2: 11-17 years	3: 4-17 years
Boys			
n	53	39	39
mean : sd	870 : 231	1233 : 430	1049 : 316
range	446 - 1523	561 - 2796	590 - 2627
Girls			
n	40	35	27
mean : sd	724 : 160	788 : 216	728 : 176
range	474 - 1200	427 - 1368	441 - 1378

Table 5.1.8
Number of subjects with a mean calcium intake below the RDI and 0.7 RDI for age, for the number of occasions of nutrient measurement during the three epochs 1 (4 to 11 years), 2 (11 to 17 years) and 3 (4 to 17 years).

		0	1	2	3	4	5	6	7
Epoch 1									
Boys	<RDI	8	15	14	11	6			
	<0.7RDI	36	9	7	2	0			
Girls	<RDI	1	6	5	13	15			
	<0.7RDI	14	11	10	4	1			
Epoch 2									
Boys	<RDI	11	6	12	5	5			
	<0.7RDI	23	10	3	1	2			
Girls	<RDI	1	3	7	11	13			
	<0.7RDI	6	10	11	7	1			
Epoch 3									
Boys	<RDI	3	4	7	9	4	5	3	4
	<0.7RDI	17	12	3	3	2	1	1	0
Girls	<RDI	0	1	1	4	3	3	6	9
	<0.7RDI	4	0	10	4	5	3	0	1

5.1.4 Nutrient intake and bone status

Univariate correlation between bone variables (BMC, BW, aBMD, vBMD) at each age and nutrient intake at the same age, in each sex, indicated few significant correlations between nutrient intake and bone with the majority of r values in the range -0.25 to 0.25. Nutrient variables tested were energy and calcium, protein, sodium, phosphorus (11 and 17 years only), vitamin D and fibre as absolute intakes and as the ratios of calcium to energy, protein, sodium, phosphorus and fibre (Tables 5.1.9, 5.1.10). There were no consistently significant correlations across ages and few correlations where $p<0.02$. The only nutrients or ratios for which correlations were significant at more than one age were absolute intakes of protein

Table 5.1.9
Univariate correlation coefficients between nutrient variables and bone variables at each age in boys (b) and girls (g).

		BMC		BW		aBMD		vBMD	
		b	g	b	g	b	g	b	g
calcium	11	0.07	-0.10	0.14	0.08	-0.08	-0.19	-0.20	-0.14
	13	0.25[a]	-0.18	0.27[a]	0.00	0.19	-0.20	-0.02	-0.14
	15	0.03	-0.13	0.09	-0.20	-0.02	-0.02	-0.10	0.08
	17	0.15	0.20	0.17	0.18	0.08	0.09	-0.03	-0.05
energy	11	0.15	0.20	0.16	0.28[a]	0.06	0.03	-0.05	-0.13
	13	0.09	-0.01	0.04	0.24	0.14	-0.13	0.10	-0.23
	15	0.11	-0.01	0.14	0.08	0.05	-0.02	-0.05	-0.11
	17	0.12	0.27	0.13	0.11	0.07	0.24	0.01	0.16
protein	11	0.17	-0.01	0.22	0.10	0.00	-0.09	-0.16	-0.10
	13	0.37[b]	-0.04	0.33[b]	0.19	0.30[a]	-0.13	0.04	-0.20
	15	0.15	-0.02	0.20	0.02	0.07	-0.04	-0.06	-0.06
	17	0.37[b]	0.21	0.27[a]	0.09	0.32[a]	0.20	0.16	0.15
sodium	11	0.20	-0.01	0.22	-0.03	0.06	0.01	-0.11	0.02
	13	0.09	-0.06	0.05	0.00	0.09	-0.08	0.05	-0.10
	15	0.14	-0.13	0.23[a]	-0.10	0.03	-0.10	-0.10	-0.03
	17	0.04	-0.18	0.11	-0.02	-0.03	-0.25	-0.10	-0.16
fibre	11	0.25[a]	-0.08	0.19	0.07	0.20	-0.15	0.05	-0.14
	13	0.22	0.06	0.21	0.01	0.17	0.08	0.00	0.07
	15	0.22	0.09	0.25[a]	0.19	0.14	0.00	0.00	-0.09
	17	0.24	0.01	0.09	0.01	0.27[a]	-0.01	0.21	-0.01
vitamin D	11	-0.06	-0.03	-0.07	0.05	0.00	-0.07	0.06	-0.05
	13	0.17	-0.11	-0.01	-0.08	0.32b	-0.08	0.37b	-0.05
	15	0.14	0.22	-0.01	0.04	0.22	0.24	0.25[a]	0.19
	17	-0.23	-0.10	0.03	0.01	0.30[a]	-0.17	0.27[a]	-0.15
phosphorus	11	0.12	-0.07	0.20	0.09	-0.08	-0.16	-0.20	-0.15
	17	0.18	0.16	0.15	0.07	0.14	0.14	0.04	0.08

a: $p<0.05$, b: $p<0.01$

Table 5.1.10
Univariate correlation coefficients between nutrient ratios and bone variables at each age in boys (b) and girls (g)

Nutrient		BMC		BW		aBMD		vBMD	
		b	g	b	g	b	g	b	g
ca/kj	11	0.01	-0.21	0.09	-0.12	-0.11	-0.17	-0.19	-0.06
	13	0.25[a]	-0.19	0.30[a]	-0.13	0.12	-0.15	-0.10	-0.07
	15	-0.06	-0.15	0.03	-0.31[a]	-0.10	0.02	-0.15	0.12
	17	0.13	0.08	0.17	0.17	0.06	-0.04	-0.06	-0.18
ca/protein	11	-0.06	-0.11	0.00	-0.03	-0.11	-0.12	-0.14	-0.07
	13	0.07	-0.22	0.10	-0.16	0.03	-0.18	-0.07	-0.10
	15	-0.08	-0.17	-0.02	-0.31[a]	-0.08	-0.01	-0.10	-0.20
	17	-0.07	0.09	0.04	0.13	-0.13	0.01	-0.17	-0.15
ca/na	11	-0.07	-0.08	-0.02	0.12	-0.12	-0.20	-0.12	-0.19
	13	0.23	-0.17	0.25a	-0.01	0.14	-0.18	-0.05	-0.14
	15	-0.05	-0.02	-0.04	-0.13	-0.03	0.06	-0.07	0.09
	17	0.09	0.32[a]	0.11	0.12	0.04	0.33[a]	-0.04	0.11
ca/fibre	11	-0.18	-0.01	-0.05	0.03	-0.28a	-0.05	-0.21	-0.06
	13	0.03	-0.19	-0.14	-0.32[a]	-0.17	0.03	-0.09	0.17
	15	-0.19	-0.14	-0.14	-0.32[a]	-0.17	0.03	-0.09	0.17
	17	-0.09	0.24	0.12	0.23	-0.23	0.13	-0.27	-0.06
ca/phos	11	0.00	-0.08	0.05	-0.01	-0.06	-0.11	-0.15	-0.10
	17	0.04	0.18	0.18	0.28[a]	-0.07	0.00	-0.18	-0.22

a: $p<0.05$

with BMC, BW and aBMD (13 and 17 years), vitamin D with aBMD (13 and 17 years) and vBMD (13, 15 and 17 years) in boys, and calcium/fibre ratio with BW (13 and 15 years) in girls. A similar analysis was performed using the mean calcium intake for each epoch and bone variables at 11, 17 and 17 years respectively. There were no significant correlations (Table 5.1.11). The power to detect a significant correlation of 0.30 ranged from 60 to 70% depending on the precise sample size at each age.

Table 5.1.11
Univariate correlation coefficients between the mean calcium intake in each epoch and bone variables at the end of the epoch: Epoch 1 (4-11 years), Epoch 2 (11-17 years), Epoch 3 (4-11 years), in boys (b) and girls (g).

	BMC		BW		aBMD		vBMD	
	b	g	b	g	b	g	b	g
Epoch 1[1]	0.08	0.02	0.16	0.22	-0.10	-0.12	-0.22	-0.21
Epoch 2[2]	0.12	0.01	0.18	0.02	0.04	0.00	-0.07	-0.01
Epoch 3[2]	0.20	0.05	0.16	0.19	0.17	-0.09	0.08	-0.21

1: bone variables at 11 years
2: bone variables at 17 years

Bone variables at the end of each epoch were compared between subjects consistently at the extremes of habitual dietary calcium intake (less than 0.7 RDI, greater than RDI) in each epoch ie epoch 1: bone variables at age 11, epochs 2 and 3: bone variables at age 17 years. Results are shown in Table 5.1.12. Although calcium intakes were significantly different in the two groups in each epoch and in both sexes ($p<0.001$) there were no statistically significant differences between the groups in any bone variables. Due to the small number of subjects who fell into these extreme intakes the power to detect a significant difference in the bone variables was less than 25%.

In order to correct for energy intake, body size, and confounding nutrients such as protein intake, the analysis was repeated comparing the top and bottom quintiles for calcium density, calcium per cm body length, calcium per kilogram body mass, and calcium to protein ratio. There were no significant differences in bone variables between the two groups in any of the three epochs. A similar analysis was performed for protein and vitamin D intakes, selecting those subjects with intakes consistently in the top and bottom quintiles for the 4 measures in epoch 2. As very few subjects fell into these categories more than twice, comparison of bone variables at age 17 was made between those whose intake was in the top quintile twice or more and those whose intake was in the bottom quintile twice or more. There were no

Table 5.1.12
Mean values of bone variables at the end of each epoch in subjects with a consistently high (>RDI) or low (<0.7RDI) calcium intake during the epochs 4-11 years (Epoch 1), 11-17 years (Epoch 2), with 4 occasions of measurement in each epoch, and 4-17 years (Epoch 3), with 7 occasions of measurement, and the mean and range of calcium intakes of all measurements in each epoch.

		n	Calcium mg mean	range	BMC g/cm	BW cm	aBMD g/cm^2	vBMD g/cm^3
Epoch 1								
Boys	4 times >RDI	8	1252	1020-1523	0.71	2.45	0.29	0.33
	2-4 times<0.7RDI	9	595	446-730	0.72	2.36	0.30	0.36
Girls	3-4 times >RDI	7	979	876-1200	0.68	2.49	0.27	0.29
	3-4 times <0.7RDI	5	500	474-520	0.70	2.41	0.29	0.33
Epoch 2								
Boys	4 times >RDI	11	1679	1249-2796	1.43	3.30	0.43	0.42
	3 -4 times<0.7RDI	3	635	561-681	1.15	2.87	0.40	0.43
Girls	3-4 times >RDI	4	1165	920-1368	1.27	2.95	0.43	0.45
	3-4 times <0.7RDI	8	581	426-625	1.21	2.84	0.43	0.46
Epoch 3								
Boys	6-7 times >RDI	7	1516	1216-2063	1.53	3.26	0.47	0.46
	4-6 times<0.7RDI	4	633	589-729	1.36	3.10	0.44	0.44
Girls	4-7 times >RDI	6	989	816-1175	1.18	2.90	0.41	0.43
	4-7 times <0.7RDI	9	580	456-672	1.21	2.82	0.43	0.46

significant differences between groups in any of the bone variables, although the means for nutrient intakes were of course significantly different. As in the analysis for absolute calcium intake, the power to detect a significant difference was less than 25%.

5.1.5 Nutrient intake and rate of change in bone status

There were very few significant univariate correlations between the change in bone variables and mean nutrient intake (initial and final ages), in each two-year interval (Table 5.1.13). The only significant correlations were at $p<0.05$ and there were no significant correlations between any bone variable and any nutrient at more than one of the three intervals. A similar analysis of change in bone variables from 11 to 17 years and nutrient intake (as the mean of the four diet records) showed only one significant correlation (energy intake and vBMD in girls) (Table 5.1.13). Top and bottom quintiles of the mean of the four measures of calcium intake, calcium per cm body length, calcium per kilogram body mass, calcium density and calcium/protein ratio were related to changes in bone variables from age 11 to 17 years. There were no significant differences between any two groups in any of these variables (Table 5.1.14).

Subjects were selected according to the number of times their calcium intake was greater than RDI or < 0.7 RDI and the changes in bone variables from 11 to 17 years in the two groups compared. Changes in all bone variables were greater in the high intake group than the low intake group. These differences were not significant in boys but they were in girls in BMC, aBMD and vBMD (Table 5.1.15).

Comparison of age at menarche, bone age at 17 years and rate of change in height from 11 to 17 years between the high and low intake groups in girls showed there were no significant differences in any of these variables (age at menarche 13.4, 13.5years; bone age 17 years 16.8 16.9 years; Δ height 24.6, 22.4 cm/6years in high and low intake groups respectively).

Table 5.1.13
Univariate correlation coefficients between mean nutrient variables (initial and final values) and rate of change of bone variables in each two-year interval and mean nutrient variables (mean of 4 values) and rate of change of bone variables in the six-year interval, in boys (b) and girls (g).

	Interval	ΔBMC		ΔBW		ΔaBMD		ΔvBMD	
		b	g	b	g	b	g	b	g
calcium	11-13	0.25[a]	-0.07	0.26[a]	0.03	0.15	-0.06	0.04	-0.04
	13-15	-0.03	-0.07	0.04	0.04	-0.02	-0.08	-0.04	-0.12
	15-17	-0.08	0.24	-0.01	0.11	-0.04	0.16	0.01	0.20
	11-17	0.07	0.24	0.05	0.08	0.03	0.22	0.22	0.12
protein	11-13	0.20	0.09	0.19	0.22	0.17	-0.01	0.04	-0.03
	13-15	0.02	-0.02	-0.05	-0.08	-0.03	-0.01	-0.04	-0.02
	15-17	-0.21	0.28[a]	-0.12	0.16	-0.16	0.20	-0.05	0.28
	11-17	0.23	0.26	0.03	0.02	0.14	0.27	0.14	0.21
energy	11-13	-0.02	0.15	-0.14	-0.04	0.08	0.16	0.11	0.18
	13-15	-0.07	0.04	0.07	-0.13	-0.14	0.06	-0.17	0.07
	15-17	-0.23	0.23	0.00	0.11	-0.23	0.16	-0.13	0.22
	11-17	-0.11	0.20	-0.14	-0.19	-0.11	0.28	-0.07	0.31[a]
sodium	11-13	0.07	-0.14	-0.01	0.01	0.09	-0.12	0.11	-0.12
	13-15	-0.07	-0.14	-0.04	-0.03	-0.11	-0.10	-0.11	-0.03
	15-17	-0.30[a]	0.22	-0.08	0.29[a]	-0.27[a]	0.10	-0.19	0.13
	11-17	-0.07	-0.21	-0.14	0.02	-0.08	-0.18	-0.03	-0.06
fibre	11-13	0.11	0.24	0.10	-0.06	0.05	0.26[a]	-0.02	0.21
	13-15	0.16	-0.04	-0.09	-0.06	0.14	-0.04	0.10	-0.05
	15-17	-0.14	0.06	-0.20	0.18	-0.03	-0.07	0.06	-0.02
	11-17	0.20	0.18	-0.21	-0.12	0.24	0.28	0.21	0.28
vitamin D	11-13	0.08	-0.17	0.01	-0.02	0.10	-0.15	0.05	-0.12
	13-15	0.12	-0.03	-0.01	0.18	0.11	-0.16	0.08	-0.22
	15-17	-0.31[a]	-0.01	-0.08	0.00	-0.29[a]	-0.03	-0.21	-0.03
	11-17	0.06	-0.10	0.00	0.02	0.04	-0.16	0.02	-0.15

a: $p<0.05$

Table 5.1.14
Mean values of the changes in bone variables from 11 to 17 years of subjects in the top and bottom quintiles of the mean calcium intake and calcium/height, calcium/weight, calcium/kj and calcium/protein ratios in the epoch 11-17 years.

Variable		Variable mean	ΔBMC g/cm	ΔBW cm	ΔaBMD g/cm^2	ΔvBMD g/cm^3
Boys						
calcium	>80	1778	0.72	0.81	0.14	0.08
mg	<20	690	0.70	0.78	0.15	0.09
ca/height	>80	1113	0.70	0.82	0.14	0.07
mg/m	<20	450	0.74	0.79	0.15	0.09
ca/weight	>80	36.4	0.71	0.82	0.14	0.07
mg/kg	<20	14.2	0.79	0.83	0.16	0.09
ca/kj	>80	176	0.70	0.85	0.13	0.07
mg/MJ	<20	81	0.64	0.78	0.13	0.05
ca/protein	>80	17.1	0.69	0.80	0.14	0.08
mg/g	<20	8.9	0.71	0.83	0.14	0.06
Girls						
calcium	>80	1124	0.54	0.40	0.15	0.13
mg	<20	561	0.47	0.39	0.13	0.11
ca/height	>80	688	0.54	0.40	0.15	0.13
mg/m	<20	352	0.48	0.38	0.13	0.11
ca/weight	>80	24.1	0.48	0.51[a]	0.13	0.08
mg/kg	<20	10.1	0.49	0.35	0.13	0.12
ca/kj	>80	149	0.47	0.39	0.13	0.10
mg/MJ	<20	78	0.53	0.46	0.14	0.11
ca/protein	>80	15.9	0.51	0.43	0.14	0.11
mg/g	<20	9.0	0.45	0.42	0.12	0.09

a: $p<0.05$

Table 5.1.15
Change in bone variables from 11 to 17 years in subjects with a consistently high (>RDI) or low (<0.7RDI) calcium intake in the epoch 11-17 years.

Group	No. times	N	Calcium mg	ΔBMC g/cm	ΔBW cm	ΔaBMD g/cm^2	ΔvBMD g/cm^3
Boys							
>RDI	4	11	1679	0.69	0.80	0.13	0.06
<0.7RDI	3-4	3	635	0.51	0.72	0.10	0.03
Girls							
>RDI	3-4	4	1165	0.62[b]	0.44	0.17[c]	0.15[a]
<0.7RDI	3-4	8	581	0.51	0.42	0.14	0.11

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ for difference between groups

5.2 PHYSICAL ACTIVITY

5.2.1 Physical activity score

The mean, sd, range and 10, 50 and 90th percentiles of physical activity score at each age by sex are shown in Table 5.2.1. Scores were consistently higher in boys than in girls, and in both sexes the score decreased with age between 11 and 13 to 15 years, and again from 15 to 17 years. Subjects with the lowest scores at 11 to 15 years travelled to school by bus or car, spent recess and lunchtimes sitting, had no regular after school activities and participated, at most, in one sport for half the year only. Those with the lowest scores at 17 years were not involved in any regular sport or exercise activity and were sedentary for 10 to 12 hours of the day. Those subjects with the highest activity scores at 11 to 15 years were moderately to very active at recess and lunchtime, were physically active most days after school, participated in two or more team sports all year round and were active at weekends. At 17 years those with the highest scores were involved in regular team sports with matches and training, and pursued other active pastimes. Two of the subjects with high scores at 17 years were in the workforce, one was involved in manual labour at a warehouse and the other was a sales assistant who spent the day standing and walking as well as playing a regular sport.

In order to identify those subjects with consistently high and low physical activity scores the number of times subjects had scores above or below the 80th and 20th percentiles respectively was determined. Table 5.2.2 shows the frequency distribution. Table 5.2.3 shows the tracking coefficients of physical activity score between each age. In both boys and girls there was a strong degree of tracking in the two year intervals 11-13, and 13-15 years, but not 15-17 years. The data suggest that the decline in habitual physical activity at age 17 years occurred randomly, presumably associated with lifestyle changes related to tertiary education or starting work. The individual lifestyles of those who changed most in their pattern of physical activity were not examined.

Table 5.2.1
Mean daily physical activity score in boys and girls at each age and mean score of all four scores.

Age years	n	Mean	sd	Range	Percentiles		
					20	50	80
Boys							
11	57	11.4	3.9	2.5-21	8.0	11.5	14.5
13	55	9.6	4.4	1.5-24	5.5	9.0	12.5
15	54	9.5	4.0	0.5-22	5.5	9.0	13.0
17	43	7.4	3.5	1.5-1 6	4.0	7.0	11.0
11-17	43	9.0	2.7	2.4-16.6	7.0	9.0	11.7
Girls							
11	52	9.3	4.0	2.5-19	5.0	9.5	12.5
13	48	7.1	3.0	1.0-14.5	4.0	6.5	10.0
15	48	6.8	3.0	0.5-13	4.0	7.0	9.5
17	37	5.7	4.0	0.5-16	2.0	5.0	9.5
11-17	36	7.0	2.3	2.9-12.3	5.2	6.9	8.7

Table 5.2.2
Number of subjects for each number of occasions activity score was above the 80th percentile and below the 20th percentile

	0	1	2	3	4
Boys					
>80th	24	15	0	2	2
<20th	23	12	3	3	2
Girls					
>80th	22	9	2	3	1
<20th	16	14	4	3	0

Table 5.2.3
Tracking coefficients of physical activity score in boys (right of diagonal) and girls (left of diagonal).

	11	n	13	n	15	n	17	n
11			0.70[c]	55	0.44[c]	54	0.19	43
13	0.54[c]	48			0.48[c]	54	0.34[a]	43
15	0.39[b]	47	0.65[c]	47			0.17	43
17	-0.13	36	0.01	36	-0.08	36		

a: $p<0.05$, b: $p<0.01$, c: $p<0.001$

5.2.2 Physical activity and bone status

Univariate correlation between bone variables (BMC, BW, aBMD, vBMD) at each age, and physical activity score at the same age, indicated few significant correlations with most r values in the range -0.17 to 0.27 (Table 5.2.4). The mean physical activity score of three ages (11, 13, 15 years) was compared with bone variables at age 15 years, and the mean score of all four ages was compared with bone variables at 17 years. There were no significant correlations between these average physical activity scores and the bone variables (Table 5.2.4).

Table 5.2.4
Univariate correlation coefficients between physical activity score and bone variables, at each age, mean score of 11 to 15 years (15 mean) and bone variables at 15 years and mean score of 11 to 17 years with bone variables at 17 years.

AGE	BMC		BW		aBMD		vBMD	
	b	g	b	g	b	g	b	g
11	0.26[a]	0.23	0.10	0.14	0.35[b]	0.18	0.20	0.06
13	-0.10	-0.12	-0.11	0.06	-0.03	-0.17	0.04	-0.19
15	-0.08	0.14	-0.09	0.15	-0.03	0.09	0.01	0.02
17	0.09	0.27	0.22	0.11	-0.04	0.30[a]	-0.12	0.15
11-15[1]	-0.12	0.09	-0.08	0.21	-0.09	-0.01	-0.07	-0.09
11-17[2]	0.01	0.27	0.07	0.20	-0.02	0.23	-0.06	0.03

a: $p<0.05$; b: $p<0.01$
1: bone variables at 15 years
2: bone variables at 17 years

Subjects were selected according to whether their physical activity score was in the top or bottom quintile at each age in boys and girls separately. Bone variables in the two groups were compared at each age by sex (Table 5.2.5). Similarly subjects were selected according to whether their mean score of all four activity scores was in the top or bottom quintile and bone variables at 17 years compared (Table 5.2.5). Subjects were also selected according to whether they had an activity score in the top or bottom quintile on three or four occasions and bone variables at 17 years compared (Table 5.2.5). There were no significant differences in any bone variables between any groups in boys. BMC and aBMD at 11 years were significantly greater in the top quintile than the bottom quintile for activity score at 11 years, and BMC at 17 years was significantly greater in the top quintile of mean activity score of the four ages, in girls. No other groups were significantly different in girls. The power to detect a significant difference between the means of the two extreme groups was 30%.

Table 5.2.5
Bone variables at each age of subjects in the top and bottom quintiles of physical activity score at each age; bone variables at 17 years of subjects in: the top and bottom quintiles of the mean activity score, and consistently in the top or bottom quintiles of activity score in the epoch 11-17 years, in boys and girls

Age	Group	BMC mg/cm	BW cm	aBMD g/cm^2	vBMD g/cm^3
Boys					
11	>80	0.83	2.56	0.33	0.37
	<20	0.74	2.43	0.30	0.34
13	>80	0.87	2.82	0.31	0.32
	<20	0.92	2.91	0.31	0.32
15	>80	1.18	3.09	0.38	0.38
	<20	1.21	3.17	0.38	0.36
17	>80	1.37	3.29	0.42	0.41
	<20	1.34	3.04	0.44	0.45
mean[1] 11-17	>80	1.47	3.29	0.44	0.43
	<20	1.55	3.32	0.46	0.45
consistently[1] (3-4 times)	>80	1.51	3.34	0.45	0.43
	<20	1.53	3.33	0.45	0.43
Girls					
11	>80	0.71[b]	2.49	0.29[b]	0.31
	<20	0.61	2.36	0.26	0.29
13	>80	0.84	2.79	0.30	0.31
	<20	0.89	2.77	0.32	0.33
15	>80	1.02	2.69	0.39	0.42
	<20	1.02	2.72	0.37	0.40
17	>80	1.22	2.83	0.43	0.46
	<20	1.09	2.73	0.40	0.45
mean[1] 11-17	>80	1.20[a]	2.88	0.42	0.44
	<20	1.06	2.70	0.39	0.44
consistently[1] (3-4 times)	>80	1.21	2.80	0.43	0.46
	<20	1.11	2.81	0.40	0.43

1: bone variables at 17 years

5.2.6 Physical activity and rate of change in bone status

Univariate correlation between changes in bone variables in each two-year interval and physical activity score in the same period (mean of score at initial and final ages), by sex, showed no significant associations (Table 5.2.6). There was a similar negative result between changes in bone variables from 11 to 17 years and activity score as the mean of the records (Table 5.2.6). The changes in bone variables were compared between those subjects with a consistently high and consistently low exercise score, but again there were no significant differences (Table 5.2.7).

Table 5.2.6
Univariate correlation coefficients between the mean of the initial and final physical activity scores and changes in bone variables, in each two-year interval, and between the mean of the four scores and changes from 11 to 17 years, by sex.

Interval	ΔBMC		ΔBW		ΔaBMD		ΔvBMD	
	b	g	b	g	b	g	b	g
11-13	-0.18	-0.11	-0.13	0.16	-0.17	-0.17	-0.09	-0.17
13-15	-0.22	0.07	-0.10	-0.08	-0.14	0.09	-0.09	0.09
15-17	-0.16	0.03	-0.01	-0.05	-0.18	0.03	-0.12	-0.10
11-17	-0.04	0.19	-0.02	0.03	0.06	0.13	-0.04	-0.01

Table 5.2.7
Rate of change in bone variables in those with physical activity scores consistently in the top and bottom quintiles in the epoch 11-17 years.

	N	Mean score	ΔBMC g/cm	ΔBW cm	ΔaBMD g/cm^2	ΔvBMD g/cm^3
Boys						
>80	5	14.5	0.68	0.74	0.13	0.08
<20	5	4.2	0.76	0.80	0.15	0.08
Girls						
>80	3	11.3	0.51	0.43	0.13	0.08
<20	3	3.5	0.47	0.33	0.14	0.12

DISCUSSION

5.1 Nutrient intake

In this study nutrient intake was not found to be consistently correlated with bone size, mass or density. This finding is consistent with a number of studies in children and adolescents (Glastre et al 1990, Katzman et al 1991, Nowson et al 1992) but contrasts with others (Miller and Johnston 1990, Sentipal et al 1991, Chan 1991, Turner et al 1992, Gunnes and Lehmann 1995). These discrepancies may reflect methodological error and indicate that the effects of diet and physical activity are too weak to be detected in a relatively small study.

Similarly rates of change in bone status were not significantly correlated with calcium intake a finding consistent with the 1-year prospective study of Gunnes and Lehmannn (1996). However in the present study, there was a significantly greater increase in bone mass and density from 11 to 17 years, in those girls with consistently high calcium intakes than in those with consistently low intakes. This difference could not be attributed to differences in pubertal or skeletal maturity. There were similar differences in the rates of change of bone status in boys between those with high and low calcium intakes but these were not significant. The negative results in the total sample may reflect the inconsistency of calcium intake between intervals as well as a type II error. The positive result in girls is consistent with findings from intervention studies using calcium supplements or dairy products to increase calcium intake (Nowson et al 1994, Gilchrist et al 1995).

In this study a 4-day weighed food record was the methodology used. This may give an accurate record of intake over a particular 4 days, but it does not necessarily indicate usual intake over a longer period. However this choice of method is a compromise between subjects' compliance and the need to collect food records over a sufficiently long period to represent usual intake. The variability of calcium intake over time is indicated by the degree of tracking (the amount of the variance at any one time that is determined by the intake at a previous time). In this study tracking decreased over longer time intervals ie there was inconsistency of calcium intakes over long periods.

Although in this study there are nutrient intake data spanning up to a 13 year period, and including seven measuring points, there is no way of assessing the extent to which calcium intake fluctuated between measuring points. Nonetheless, these repeated estimations must be a more accurate estimate of calcium intake over a long time than a single measure at one point, or a retrospective estimation of intake.

It must be remembered that adequacy of calcium nutrition is more than just a matter of calcium intake, the critical factor which may influence the rate of mineral accrual in bone and final peak bone mass is calcium balance, which does not necessarily equate to intake as discussed in chapter 2. It was not possible for instance to control for variations in the bioavailability of calcium, such as the source of calcium and other nutrients in the diet, or in the calcium absorption. However it is extremely unlikely that vitamin D, the major regulator of intestinal absorption of calcium, was inadequate in these subjects; the vitamin D status of the body depends on UV light and these subjects were active and free-living with more than adequate UV exposure.

Thus given the wide range of calcium intakes observed, the variability of intakes across time, the intrinsic error in estimating calcium intake, and the unknown relationship between the actual calcium intake and calcium balance, then the lack of association found between calcium intake and bone status could be partly explained by the confounding effects of methodological problems being greater than the actual variance of net calcium absorption on bone accrual. Measurement at a number of points partially addressed the problem of fluctuating intake and enabled identification of subjects at the extremes of intake. In most instances bone status and rate of change in bone status was greater in those with consistently high intakes that in those at the other extreme but the small numbers of subjects in each group and consequently low power of the significance test may have resulted in a type II error except in change of bone status in the six-year interval in girls.

The negative findings may also be a function of the age of the subjects and the site of measurement. The majority of cross-sectional and intervention studies which have

demonstrated a positive effect for calcium on bone mass and density, have been on pre-pubertal children. As a consequence, it was thought that the pre-pubertal years might be the critical time for calcium to have an effect. However two recent studies have shown a positive effect of calcium supplementation in the form of dairy products in pubertal girls. Significant increases in aBMD were demonstrated by Gilchrist et al (1995) in the hip, LS and TB and by Chan et al (1995) in the TB and LS, but in the latter study similar increases were not observed in the forearm or FN. A one-year prospective study of 470 children aged 8 to 17 years by Gunnes and Lehmann (1996) reported that calcium intake was a significant predictor of change in ultradistal and distal forearm aBMD in prepubertal children. Conversely in pubertal subjects calcium was only a significant predictor of change in aBMD in the ultradistal forearm where trabecular bone is predominant. This does not explain why differences have been observed in TB aBMD, which reflects cortical bone, but not the appendicular cortical bone sites of distal forearm and FN. None of these studies has measured bone density as vBMD. This introduces a small degree of error in assessing bone density in growing subjects. In contrast to these studies the present study has shown significant differences in rate of change of vBMD at the distal radius in pubertal girls.

What is not known from these studies is whether the bone (BMC, aBMD) gained from supplementation results in a higher peak bone status or whether the supplementation hastens the achievement of a genetically preprogrammed peak. In addition, if supplementation can result in a higher peak, is this greater bone status maintained throughout adult life until age- and menopause-related bone loss begin, and so convey a beneficial effect with respect to reducing later fracture risk? The present study is unable to answer this question.

To determine if calcium supplementation can enhance peak bone mass and density, longitudinal studies are required in which vBMD is measured in order to account fully for differences in bone size. The subjects need to be followed from late childhood through the pubertal years to skeletal maturity and into the third decade.

5.2 Physical activity

In this study physical activity was not found to be correlated with bone size, mass or density or rate of change in bone status. This finding is consistent with a number of studies in children and adolescents (Katzman et al 1991, Nowson et al 1992, Kroger et al 1993). However other studies have shown physical activity (Slemenda et al 1991b, Turner et al 1992) to be a significant predictor of bone density. As with calcium these discrepancies may reflect methodological error and indicate that the effect of physical activity is too weak to be detected in a relatively small study.

In assessing physical activity and its potential effect on bone it is not possible to determine the relative stress magnitudes or the number of loading cycles of the wide range of activities in which normal children and adolescents engage. Thus the physical activity score in the present study was a crude estimate of the mechanical load placed on the skeleton as a whole. An additional methodological problem is that it is unknown whether a strain applied at one site can affect bone at another site.

The activity levels of the subjects in the present study ranged from semi-sedentary to moderately active, with hours of weight-bearing exercise ranging from 2 to 25 per week. These levels cover the range of activities in normal apparently healthy adolescents who are not involved in elite sporting activities. Levels of activity varied from year to year with few subjects consistently having either very low levels or very high levels within the normal range. As bone was measured during the time of most rapid growth, it maybe that the individual variation in rate of change confounded any true differences in bone consequent to exercise levels. It is interesting that the studies which found differences in bone growth according to activity level involved predominantly pre-pubertal children engaged in a heavy training schedule (Slemenda et al 1991b, Slemenda and Johnston 1993, Bass et al 1995).

These findings in girls are supported by the results of a one-year prospective study in 470 boys and girls aged 8 to 16 years (Gunnes and Lehmann 1996). The authors of this study reported that weight-bearing physical activity was a significant predictor of change in aBMD of the

distal and ultradistal forearm in boys aged 11 years and over and less than 11 years but in girls weight-bearing physical activity was a significant predictor only for change in the distal forearm and only in those less than 11 years of age.

It is probable that the subjects in the present study had activity levels that were within the range which ensured normal bone growth, but not at the extreme levels which may enhance bone mass. Physical activity programmes in schools ensure all children are involved in daily activity in the early adolescent years. The effects of a reduced activity in later adolescence may mean a lower PBM is achieved but provided there is some activity the deficit is likely to be very small. Since the relation of bone density to fracture risk is a continuum any gain must be considered beneficial.

As for calcium, to determine the role of physical activity, short of elite athletic training, in determining PBM, and whether bone added to the skeleton prior to maturity as a result of enhanced mechanical loading can be retained into and throughout adulthood, well controlled longitudinal studies in which vBMD is measured and subjects are stratified according to a much wider range of physical activity and training are needed. This would clarify the role of physical activity and training in optimising bone accrual prior to skeletal maturity, when it is physiologically possible to add bone to the skeleton.

CHAPTER 6

RESULTS: BONE STATUS AND GENETIC FACTORS

6.1 INTRAFAMILIAL CORRELATIONS OF BONE VARIABLES

Values (mean and sd) of parental age, height, weight and bone variables are shown in Table 6.1. The value of vBMD for mothers, corrected for age above 55 years and years since menopause (vBMD corrected), is included.

Table 6.1
Parental values for age, height, weight and bone variables as mean and sd.

	Fathers (n=78)		Mothers (n=98)	
	mean	sd	mean	sd
Age years	47.1	5.6	44.6	4.8
Height cm	176.6	7.0	162.0	6.5
Weight kg	80.9	13.3	62.4	11.4
BMC g/cm	1.76	0.22	1.23	0.17
BW cm	3.40	0.27	2.82	0.25
aBMD g/cm^2	0.52	0.05	0.43	0.05
vBMD g/cm^3	0.49	0.05	0.45	0.06
corrected			0.48	0.05

Univariate correlation coefficients of each bone variable between sons and daughters, and mothers and fathers are shown in Table 6.2. There were significant positive correlations for BMC between sons and their mothers throughout adolescence and between daughters and mothers at 15 and 17 years, and similarly for BW. There were weaker correlations of aBMD between mothers and both sons and daughters, and generally weaker correlations again, of vBMD, but these were not significantly different from those of BMC and BW. Correlations were greatest in boys at 11 years, and least at 13 years. In girls the correlations of aBMD and vBMD with mothers increased at each age from a low at age11. Correction of mothers' values

for age and years since menopause strengthened the association between mothers and sons but made no difference to the correlation between mothers and daughters. There were no significant correlations of BMC or BW between fathers and daughters at any age. Correlations between fathers and sons were significant only at 11 and 17 years for BMC and 11 years for BW. Correlations of aBMD and vBMD between fathers and children were weaker than mothers and children and most non-significant, but there were a few at $0.02<p<0.05$.

Use of standard deviation scores in place of absolute values of parental vBMD made no difference to the correlations between mothers and fathers and their children.

Table 6.3 shows the univariate correlation coefficients of each bone variable against the mean of mother's and father's values combined. In boys, there were significant correlations between the mean of the parents' BMC and BW and the sons' variables at all ages. These correlations were weaker than the corresponding correlation between sons and mothers but not significantly. There were significant correlations between sons and their mean parental aBMD at 15 and 17 years, and mean parental vBMD at ages 11, 15 and 17. In girls, there were significant correlations with mean parental values of all bone at 15 and 17 years, and with mean parental BW and vBMD at age 13. These correlations were mostly weaker but not significantly different from those between daughters and mothers. Using mothers' corrected values of vBMD did not alter the strength of the mid-parent correlations.

Table 6.2
Univariate correlation coefficients of children's bone variables with their parents' values.

Age (years)	11	13	15	17
Mothers-sons				
n	53	52	53	51
BMC	0.46[d]	0.42[c]	0.45[c]	0.54[d]
BW	0.53[d]	0.52[c]	0.53[c]	0.52[d]
aBMD	0.37[c]	0.19	0.26	0.34[b]
vBMD	0.46[d]	0.13	0.20	0.24[a]
corrected vBMD	0.47[d]	0.13	0.26[a]	0.29[a]
Fathers-sons				
n	42	42	42	40
BMC	0.27[a]	0.17	0.23	0.28[a]
BW	0.32[a]	0.12	0.18	0.25
aBMD	0.15	0.10	0.23[a]	0.20
vBMD	0.20	0.09	0.28[a]	0.25
Mothers-daughters				
n	44	43	44	42
BMC	0.20	0.25	0.46[d]	0.56[d]
BW	0.31[a]	0.38[a]	0.41[c]	0.41[c]
aBMD	0.08	0.16	0.38[c]	0.44[c]
vBMD	0.08	0.16	0.30[a]	0.28[a]
corrected vBMD	0.07	0.16	0.31[a]	0.28[a]
Fathers-daughters				
n	34	34	34	33
BMC	0.02	0.13	0.17	0.22
BW	-0.06	0.16	0.21	0.18
aBMD	0.09	0.16	0.20	0.06
vBMD	0.07	0.35[a]	0.31[a]	0.12

a: $p<0.05$, b: $p<0.01$, c: $p<0.005$, d: $p<0.001$

The univariate correlation coefficients between mothers', fathers', and mean parental heights and sons' and daughters' heights are shown in Table 6.4. They were all highly significant, the strongest correlation being with mean parental height. As BMC is highly dependent on bone size, and parental height is a strong predictor of child's height, the positive correlation between parental BMC and child BMC could partly be accounted for by a confounding correlation between height and bone size, but correcting for parental height did not reduce the correlation coefficients.

Table 6.3
Univariate correlation coefficients of children's values with mean parental values.

Age (years):	11	13	15	17
Boys				
n	41	41	41	39
BMC	0.42^{c}	0.33^{a}	0.37^{b}	0.47^{d}
BW	0.58^{d}	0.43^{c}	0.48^{d}	0.52^{d}
aBMD	0.24	0.07	0.28^{a}	0.28^{a}
vBMD	0.39^{c}	0.12	0.34^{b}	0.36^{b}
Girls				
n	34	34	34	33
BMC	0.10	0.19	0.30^{a}	0.37^{a}
BW	0.14	0.34^{a}	0.37^{a}	0.34^{a}
aBMD	0.09	0.21	0.36^{a}	0.29^{a}
vBMD	0.15	0.39^{b}	0.44^{c}	0.33^{a}

a: $p<0.05$, b: $p<0.01$, c: $p<0.005$, d: $p<0.001$

Table 6.4
Univariate correlation coefficients between subject's height at age 17 years and their mother's and father's height, and mean parental height ($p<0.001$ for each).

	fathers' height	mothers' height	mean parental height
boys	0.48	0.42	0.56
girls	0.58	0.56	0.70

6.2 ESTIMATES OF HERITABILITY

Heritability, defined as the regression coefficient between parental and child values, was estimated for each bone variable in boys and girls. The subjects' values at 17 years were selected as being nearest to PBM. Only those subjects in whom data was available on both parents were included. Estimates of heritability are shown in Table 6.5. The estimates were consistently greater between mother and child than between father and child or mid-parent and child except for vBMD in boys where the estimate using the mid-parent value was marginally greater. Correction of mothers' vBMD for age and years since menopause increased the estimate slightly for mothers-sons only.

Table 6.5
Heritability estimates of each bone variable at 17 years based on the mothers', fathers' and mid-parent values.

	BMC	BW	aBMD	vBMD
Boys n=39				
mother	0.50^{c}	0.57^{c}	0.33^{a}	0.32^{a}
father	0.26	0.24	0.12	0.25
mid-parent	0.47^{c}	0.52^{c}	0.28^{a}	0.36^{b}
Girls n=33				
mother	0.37^{a}	0.36^{a}	0.36^{a}	0.33^{a}
father	0.22	0.18	0.06	0.12
mid-parent	0.37^{a}	0.34^{a}	0.29^{a}	0.32^{a}

a: $p<0.05$, b: $p<0.01$, c: $p<0.001$

6.3 CHILD VALUES AS A PERCENTAGE OF PARENTAL VALUES.

Table 6.6 shows the subjects' height and bone variables at age 17 years as a percentage of the equivalent variables of their same sex parents. Sons had attained 101% of their father's height and daughters 103% of their mother's height. Sons and daughters had attained slightly less of the bone width of their same sex parent, although in each case it was greater than 95%. In comparison boys had attained only 85% of their father's BMC whilst girls had attained 95 % of their mother's BMC. Differences in the same direction between boys and girls were also true of aBMD and vBMD, with the boys having attained less than 90% of their father's BMD and girls over 95% of their mother's BMD. These differences reflect the later increase in BMD in boys as described earlier.

Table 6.6
Height and bone variables at 17 years as a percentage (mean and range) of same sex parent's height and corresponding bone values.

Variable	**Fathers-sons**		**Mothers-daughters**	
	mean	range	mean	range
Height	101	93-110	103	95-128
BMC	85	64-126	95	66-133
BW	97	64-119	99	82-117
aBMD	87	68-119	97	73-133
vBMD	89	67-124	98	66-155

DISCUSSION

6.1 Intrafamilial correlations

The present study shows that there is a greater correlation between the bone mass of adolescents of both sexes with their mothers than with their fathers. Most previous studies which have investigated parent-child relationships have been restricted to mother-daughter pairs (Lutz 1986, Tylavsky et al 1989, Henderson et al 1995). The daughters in these studies were 18 years or older. Correlation coefficients for BMC, BW and aBMD at the mid and distal radius, spine, proximal femur and FS ranged from 0.34 to 0.50, very similar to the mother-daughter correlations at 17 years in the present study.

Matkovic et al (1990) measured bone at two radial sites and the spine in 31, 14-year-old girls, 30 of their mothers and 24 of their fathers. They reported r values of 0.46 for aBMD between mother and daughter at all three sites but in father-daughter comparisons values were 0.28 in the predominantly cortical radius and 0.57 and 0.45 at the mixed trabecular and cortical sites of the radius and spine respectively. The strongest correlations were reported at the cortical radius (r=0.52), and the cortical and trabecular radius (r=0.72) between daughters and the mid-parental values . These values are similar to those between mothers and daughters in the present study, but higher than those between fathers and daughters. They contrast with the weaker correlation of aBMD between daughters and midparental values compared with mothers' in the present study.

Krall and Dawson-Hughes (1993) studied adult members of 40 families. Each family included mother, father, son and daughter in whom the mean ages were 60, 63, 32 and 31 years respectively. aBMD of the radius was measured at the two-thirds distal site. They found stronger correlations between daughters and mothers and fathers (r 0.35 and 0.40) than between sons and their mothers and fathers (r 0.27 and 0.23) although these values are not significantly different. Correlations were greater between daughters and mid-parental values (r 0.47) than between daughters and parents separately, but correlations were similar between sons and mid-parental values (r 0.27) and parents separately. These results contrast with the

present study in which correlations between fathers and children were much weaker than between mothers and both sons and daughters. Similarly in this study the correlations of children's values with mid-parental aBMD were weaker than with mothers but greater than with fathers.

McKay et al (1994) measured aBMD at the proximal femur and LS in 41 mother-daughter pairs (mean age 39 and 12 years respectively) and 42 mother-son pairs (mean age 41 and 13 years respectively). They reported stronger correlations between mothers' and daughters' than between mothers' and sons' LS aBMD (0.34 v 0.25) and the same was true of the proximal femur (0.36 v 0.26). They suggested that the weak relationship between boys' and mothers' bone status may have been due to the immaturity of the boys. The observed variability in boys aBMD at mid-puberty, and the weaker correlations between boys and mothers at 13 and 15 years in the present study, support this hypothesis.

Although the correlation coefficients between parents and their children vary from study to study, these values are not significantly different from each other, probably due to the overall weak associations ($r<0.50$) and the small sample numbers. Differences in trends with respect to the strength of the correlations between parents and their children may be due to cohort effects ie differences between studies in the age of the subjects. These varying results may also reflect differences in the degree of shared environment of subjects in the individual studies. Correlations between family members measure overall familial resemblance which is the sum of both a common environment and genetics. Thus differences between parent-child correlations may be due to differences in exposure to a common environment.

Studies on MZ and DZ twins by Smith et al (1973), Christian et al (1990) and Slemenda et al (1991a) suggested that environmental factors had a strong influence on bone and that there were complex gene-environment interactions. Christian et al (1990) also concluded that most genetic influence on adult bone was related to inherited variations in bone size and that hereditary factors exerted their influence during growth and early adulthood.

This proposed mechanism of genetic influence on bone size is in part supported by the results of the present study which show a strong correlation between mothers' and childrens' BW, but a much weaker correlation between fathers' and children's BW despite the strong correlations between fathers' and both sons' and daughters' height . BMC which is dependent on bone size is also related in parents and children, but the variables of bone density, particularly vBMD, are less dependent on bone size and how much weaker associations. Why fathers appear to exert less influence on their children's bone size and hence bone mineral content is unclear.

6.2 Estimates of heritability

Heritability estimates are unique to each population sample studied. With increasing age it is proposed that an individual's environment exerts greater influence on bone and the familial component becomes less evident. This may be especially important in women in whom rapid bone loss after menopause is a significant source of variability in bone density. If bone density of parents and children could be measured at or near PBM, the heritability estimates would probably be different. In the present study, heritability estimates were calculated using child values at 17 years. They were generally greater in mother-child comparisons than in either father-child or in mid-parental-child comparisons. Heritability estimates were also greater in mothers-sons than in mothers-daughters, and greater in BMC and BW than in aBMD and vBMD. These values are comparable with heritability estimates reported by others. For example, Lutz (1986) estimated heritability as twice the regression coefficient of mothers on daughters. She reported heritability of BMC and aBMD of the radius, in 26 daughters aged 26 $\pm$ 5 years and their mothers, as 0.72 and 0.57 respectively. Tylavsky (1989) in a study of 18 women aged 18 to 22 years and their mothers, reported heritability estimates of distal radial BMC, BW and aBMD to be 0.49, 0.42 and 0.29 respectively. Krall and Dawson-Hughes (1993) studied families including a mother, father, son and daughter. Using the mid-parental and mid-offspring values, they estimated heritability of aBMD in TB, radius, spine and FN to be 0.69, 0.51, 0.50 and 0.70 respectively. No studies have previously reported heritability estimates based on fathers' or sons' values alone.

6.3 Child values as a percentage of parental values

In the present study, 17 year-old boys had attained 101% and 97% of their fathers' height and BW respectively, but less than 90% of BMC, aBMD and vBMD. By contrast 17 year-old girls had attained 103% of their mothers' height and 95% or greater of each bone variable. Correcting mothers's vBMD for age and years since menopause decreased the daughters' vBMD from 98 to 97% of their mothers'.

Several previous studies have compared BMC and BMD in daughters and mothers as an indication of the relation between bone of post-pubescent girls and adult women and thus of the timing of bone accrual cessation. Matkovic et al (1990) reported the height of 16 year old daughters to be comparable to that of their mothers' (99-100%), but that aBMD of the distal radius was 91% and the mid-radius was 94% of their mothers'. Tylavsky et al (1989) also reported that at both distal and mid-radial sites, 19 year-old daughters' BMC and aBMD were 10% and 5% respectively less than their 44-year-old mothers. These values are comparable to those in the present study of 17-year-old daughters and 45-year-old mothers. Henderson et al (1995) reported that 18-year-old women had attained between 103 and 110% of their mothers' aBMD at proximal femur sites, although only 98% at the spine. These studies, including the present one, suggest that BMC, aBMD and vBMD at most sites in women, continue to increase in the third decade although very slowly. No comparable published values are available for sons and fathers, but the data in the present study suggest that up to 15% of BMC and 11% of vBMD may be accrued in the third decade.

CHAPTER 7

RESULTS: RELATIONSHIP OF ALL FACTORS TO BONE STATUS

7.1 ABSOLUTE BONE STATUS

Multivariate regression analysis was performed to determine which of the various biological, environmental and genetic factors were significant predictors of bone size, mass and density. For each of the four bone variables, the independent variables were age, sex, pubertal stages 2 to 5 (PHS2 to 5), height, weight, total skinfold thickness, physical activity score, daily energy, calcium and protein intakes and the corresponding bone variable of mother and father. The variables sex and PHS2 to PHS5 were created as dummy variables, i.e. if sex was male sex = 0, if sex was female sex = 1; if PHS was 2, PHS2 = 1 and all other cases PHS2=0; if PHS was 3, PHS3=1 and all other cases PHS3=0 etc.

All variables were entered in the initial equation and non-significant variables were then eliminated one at a time until only significant variables remained. The details of the analyses are shown in Table 7.1, namely the coefficients (B), the standard error of each coefficient (se B), the t values for each variable, the adjusted R^2 (the proportion of the variation explained by the regression model taking into account the degrees of freedom), and the standard errors of the regression lines.

80% of the variance of BMC was accounted for by age, sex, PHS4, PHS5, weight, skinfold thickness, protein intake and mother's and father's BMC. The standard error of the regression line was 0.133. The equation was

BMC (g/cm) = -0.029 -0.062 (sex) + 0.067 (PHS4) + 0.103 (PHS5) + 0.040 (age)
+ 0.010 (weight) - 0.352 (skinfold) + 0.001 (protein) + 0.103 (father's BMC)
+ 0.140 (mother's BMC).

Table 7.1
Details of multiple regression analysis for each bone variable

BMC (g/cm) Adjusted $R^2 = 0.800$, standard error = 0.133

Variable	Sex	PHS 4	PHS 5	Age	Weight	Total skinfold [a]	Dietary protein	Father's BMC	Mother's BMC	Constant
Units	male = 0 female = 1	PHS 4 = 1 else = 0	PHS 5 = 1 else = 0	years	kg	mm	g/day	g/cm	g/cm	
B	-0.062	0.066	0.103	0.040	0.010	-0.352	0.001	0.113	0.140	-0.029
se B	0.022	0.025	0.032	0.007	0.001	0.066	0.0004	0.037	0.055	0.163
t	2.84	2.59	3.27	5.56	8.20	5.31	3.50	3.04	2.56	0.18

a: $\log_{10}$

BW (cm) Adjusted $R^2 = 0.714$, standard error = 0.200

Variable	Sex	PHS3	Age	Height	Weight	Dietary protein	Father's BW	Mother's BW	Constant
Units	male = 0 female = 1	PHS 3 = 1 else = 0	years	cm	kg	g/day	cm	cm	
B	-0.207	0.150	0.025	0.008	0.007	0.0014	0.184	0.334	-0.906
se B	0.029	0.032	0.010	0.002	0.001	0.0006	0.046	0.054	0.289
t	7.19	4.73	2.57	4.45	5.03	2.47	4.00	6.16	3.13

Table 7.1 continued

aBMD (g/cm^2) Adjusted R^2 =0.720, standard error = 0.037

Variable	PHS4	PHS5	Age	Height	Weight	Total skinfold [a]	Protein intake	Father aBMD	Mother aBMD	Constant
Units	PHS 4 = 1 else = 0	PHS 5 = 1 else = 0	years to 0.1	cm	kg	mm	g/day	g/cm^2	g/cm^2	
B	0.033	0.050	0.012	-0.0009	0.002	-0.089	0.0002	0.083	0.189	0.213
se B	0.007	0.009	0.002	0.0004	0.0004	0.018	0.0001	0.043	0.052	0.071
t	4.82	5.71	5.95	2.36	4.68	4.94	2.52	1.91	3.60	2.99

a: $\log_{10}$

vBMD (g/cm^3) Adjusted R^2 = 0.487, standard error = 0.046

Variable	Sex	PHS2	PHS3	Age	Height	Weight	Total skinfold [a]	Father's vBMD	Mother's vBMD	Constant
Units	male = 0 female = 1	PHS 2 = 1 else= 0	PHS 3 = 1 else= 0	years	cm	kg	mm	g/cm^3	g/cm^3	
B	0.019	-0.026	-0.045	0.017	-0.0016	0.0011	-0.087	0.141	0.256	0.292
se B	0.007	0.008	0.007	0.002	0.0005	0.0005	0.026	0.050	0.053	0.090
t	2.75	3.16	6.15	7.51	3.18	2.26	3.30	2.83	4.80	3.23

a: $\log_{10}$

71% of the variance of BW was accounted for by age, sex, PHS3, height, weight, mother's and father's BW and dietary protein. The standard error of the regression line was 0.200. The equation was

$$\begin{aligned}\text{BW (cm)} = &-0.906 - 0.207\ (\text{sex}) + 0.150\ (\text{PHS3}) + 0.025\ (\text{age}) + 0.008\ (\text{height}) \\ &+ 0.007\ (\text{weight}) + 0.184\ (\text{father's BW}) + 0.334\ (\text{mother's BW}) \\ &+ 0.0014\ (\text{dietary protein}).\end{aligned}$$

72% of the variance of aBMD was accounted for by age, PHS4, PHS5, height, weight, skinfold thickness and mother's and father's aBMD. The standard error of the regression line was 0.037. The equation was

$$\begin{aligned}\text{aBMD (g/cm}^2) = &\ 0.264 + 0.032\ (\text{PHS4}) + 0.050\ (\text{PHS5}) + 0.012\ (\text{age}) - 0.0009\ (\text{height}) \\ &+ 0.002\ (\text{weight}) - 0.108\ (\text{skinfold}) + 0.0002\ (\text{protein}) \\ &+ 0.071\ (\text{father's aBMD}) + 0.174\ (\text{mother's aBMD}).\end{aligned}$$

49% of the variance of vBMD was accounted for by age, sex, PHS2, PHS3, height, weight, skinfold thickness and mother's and father's vBMD. The standard error of the regression line was 0.046. The equation was

$$\begin{aligned}\text{vBMD (g/cm}^3) = &\ 0.292 + 0.019\ (\text{sex}) - 0.026\ (\text{PHS2}) - 0.045\ (\text{PHS3}) + 0.017\ (\text{age}) \\ &- 0.0016\ (\text{height}) + 0.0012\ (\text{weight}) - 0.087\ (\text{skinfold}) \\ &+ 0.141\ (\text{father's vBMD}) + 0.256\ (\text{mother's vBMD}).\end{aligned}$$

Each variable made a positive contribution to the variance of BMC, except sex and skinfold thickness which were negative, the former because girls have lower values than boys and the latter because girls have greater skinfold thickness than boys at all ages. Every variable was positively related to BW except sex, once again indicating the greater values in boys than girls. Height and skinfold thickness were negatively related to aBMD while every other variable was positively related. The negative association with skinfold thickness probably reflects sex

differences, while the negative association with height shows that aBMD is in part determined by bone size. aBMD was lower in those who had grown most, in particular the boys. Sex made a positive contribution to vBMD whereas early to mid PHS (PHS2, PHS3) made a negative contribution. Thus girls have greater vBMD values than boys and those in the early to mid stages of pubertal development have lower values than those at later stages. A similar effect of pubertal stage on the other bone variables is indicated by the positive effect of later PHS (PHS4, PHS5). The negative associations of height and skinfold thickness with vBMD are similar to the associations with aBMD, indicating that taller and fatter subjects have lower vBMD, although the positive contribution of weight tempers these associations. The contribution of these anthropometric measures to the variance of aBMD and vBMD probably reflect the effect of gender on bone.

Mother's and father's bone variables had positive effects on all four bone variables indicating the strong genetic component in bone.

Neither physical activity score nor calcium intake was included in any of the models. To confirm that these environmental factors were not significant predictors of bone mass and that the biological variables of height, skinfold and sex were not significant predictors of BMC, BW and aBMD respectively, each variable was forced into the final model. These new models are shown in Table 7.2. Neither calcium intake, physical activity nor height was a significant predictor of BMC, and addition of each of these to the model did not increase the adjusted R^2. The result was similar for BW and the variables calcium intake, physical activity and skinfold thickness; none of these were significant predictors, and their addition altered the adjusted R^2 by 0.5% or less. Addition of calcium intake, physical activity and sex increased the adjusted R^2 of aBMD by 0.1% and 0.6% and zero respectively. None of these variables was a significant predictor. Addition of calcium intake and physical activity to the vBMD equation resulted in a 0.7% increase in adjusted R^2 and very minor changes to the coefficients of each of the original variables in the equations. Neither variable was a significant predictor of vBMD.

Table 7.2
Details of the original multiple regression analysis and each analysis resulting from forced entry of each additional variable
For each variable: B (top line), se B (second line), t (third line)

BMC (g/cm)

Variable	Units	Original equation	Forced entry variable		
			Calcium mg/day	Physical activity score	Height cm
			-0.00001 0.00002 0.56	0.003 0.002 1.44	-0.00005 0.0015 0.03
Sex	male = 0 female = 1	-0.062 0.022 2.84	-0.064 0.022 2.88	-0.061 0.022 2.72	-0.062 0.023 2.75
PHS4	PHS 4 = 1 else = 0	0.066 0.025 2.59	0.067 0.026 2.61	0.070 0.026 2.72	0.066 0.026 2.57
PHS5	PHS 5 = 1 else = 0	0.103 0.032 3.27	0.104 0.032 3.25	0.104 0.032 3.25	0.103 0.032 3.21
Age	years	0.040 0.007 5.56	0.040 0.007 5.51	0.044 0.008 5.75	0.041 0.008 5.23
Weight	kg	0.010 0.001 8.20	0.010 0.001 8.17	0.009 0.001 7.81	0.010 0.001 6.35
Total skinfold[a]	mm	-0.352 0.066 5.31	-0.352 0.066 5.30	-0.330 0.068 4.87	-0.354 0.077 4.59
Dietary protein	g/day	0.001 0.0004 3.50	0.0015 0.0005 3.08	0.0013 0.0004 3.33	0.0014 0.0004 3.48
Father's BMC	g/cm	0.113 0.03 3.04	0.109 0.038 2.88	0.105 0.038 2.80	0.113 0.037 3.03
Mother's BMC	g/cm	0.140 0.055 2.56	0.142 0.055 2.59	0.163 0.056 2.91	0.140 0.055 2.55
Constant		-0.029 0.163 0.18	-0.020 0.164 0.12	-0.135 0.175 0.77	-0.023 0.264 0.09
Adjusted R^2 std error		0.800	0.800 0.133	0.800 0.133	0.800 0.133

a:$\log_{10}$

Table 7.2 continued

BW (cm)

Variable	Units	Original equation	Forced entry variables		
			Calcium mg/day	Physical activity score	Total skinfold[a] mm
			-0.029×10^{-6} 0.037×10^{-3} 0.001	0.006 0.003 1.85	-0.086 0.116 0.74
Sex	male = 0 female = 1	-0.207 0.029 7.19	-0.207 0.029 7.13	-0.191 0.029 6.51	-0.199 0.034 5.85
PHS3	PHS 3 = 1 else = 0	0.150 0.032 4.73	0.150 0.032 4.69	0.153 0.032 4.82	0.145 0.032 4.58
Age	years	0.025 0.010 2.57	0.025 0.010 2.57	0.031 0.010 3.11	0.024 0.010 2.41
Height	cm	0.008 0.002 4.45	0.008 0.002 4.43	0.008 0.002 4.34	0.008 0.002 3.58
Weight	kg	0.007 0.001 5.03	0.007 0.001 5.01	0.007 0.001 4.94	0.008 0.002 3.47
Father's BW	cm	0.184 0.046 4.00	0.184 0.047 3.93	0.170 0.046 3.69	0.207 0.054 4.30
Mother's BW	cm	0.334 0.054 6.16	0.334 0.056 6.00	0.347 0.055 6.33	0.327 0.054 6.00
Dietary protein	g/day	0.0014 0.0006 2.47	0.0014 0.0008 1.90	0.0013 0.0006 2.27	0.0012 0.0006 2.12
Constant		-0.906 0.289 3.13	-0.906 0.290 3.12	-0.994 0.293 3.39	-0.806 0.441 1.83
Adjusted R^2 std error		0.714 0.200	0.713 0.200	0.715 0.199	0.719 0.200

a:$\log_{10}$

Table 7.2 continued

aBMD ($g/cm^{2)}$

Variable	Units	Original equation	Forced entry variables		
			Calcium mg/day	Physical activity score	Sex male = 0 female = 1
			$-0.042x10^{-4}$ $0.065x10^{-4}$ -.65	0.0009 0.0006 1.52	-0.0025 0.006 0.41
PHS4	PHS 4 = 1 else = 0	0.033 0.007 4.82	0.034 0.007 4.84	0.035 0.007 5.10	0.034 0.007 4.82
PHS5	PHS 5 = 1 else = 0	0.050 0.009 5.71	0.050 0.009 5.73	0.050 0.009 5.80	0.050 0.009 5.70
Age	years	0.012 0.002 5.95	0.012 0.002 5.89	0.013 0.002 6.08	0.012 0.002 5.95
Height	cm	-0.0009 0.0004 2.36	-0.0009 0.0004 2.39	-0.0009 0.0004 2.29	-0.0009 0.0004 2.18
Weight	kg	0.002 0.0004 4.68	0.002 0.0004 4.71	0.002 0.0004 4.48	0.002 0.0004 4.19
Total skinfold[a]	mm	-0.089 0.018 4.94	-0.091 0.018 4.98	-0.081 0.018 4.41	-0.085 0.021 4.03
Dietary protein	g/day	0.0002 0.0001 2.52	0.0003 0.0001 2.29	0.0002 0.0001 2.47	0.0002 0.0001 2.12
Father's aBMD	g/cm^2	0.083 0.043 1.91	0.081 0.044 1.85	0.077 0.043 1.80	0.083 0.043 1.90
Mother's aBMD	g/cm^2	0.189 0.052 3.60	0.185 0.053 3.51	0.216 0.053 4.09	0.186 0.053 3.52
Constant		0.213 0.071 2.99	0.220 0.072 3.05	0.173 0.074 2.35	0.206 0.074 2.80
Adjusted R^2 std error		0.720 0.037	0.721 0.036	0.726 0.036	0.720 0.036

a:$\log_{10}$

Table 7.2 continued

vBMD (g/cm$^{3)}$

Variable	Units	Original equation	Forced entry variables	
			Calcium mg/day	Physical activity score
			-0.065×10^{-5} 0.616×10^{-5} 0.11	0.0004 0.0007 0.55
Sex	male = 0 female = 1	0.019 0.007 2.75	0.018 0.007 2.42	0.015 0.007 2.18
PHS2	PHS 2 = 1 else = 0	-0.026 0.008 3.16	-0.027 0.008 3.30	-0.028 0.008 3.49
PHS3	PHS 3 = 1 else = 0	-0.045 0.007 6.15	-0.045 0.007 6.09	-0.048 0.007 6.61
Age	years	0.017 0.002 7.51	0.017 0.002 7.56	0.016 0.002 7.14
Height	cm	-0.0016 0.0005 3.18	-0.0016 0.0005 3.27	-0.0015 0.0005 3.08
Weight	kg	0.0011 0.0005 2.26	0.0011 0.0005 2.32	0.0011 0.0005 2.20
Total skinfold[a]	mm	-0.087 0.026 3.30	-0.086 0.026 3.26	-0.074 0.026 2.85
Father's vBMD	g/cm^3	0.141 0.050 2.83	0.137 0.050 2.74	0.135 0.050 2.83
Mother's vBMD	g/cm^3	0.256 0.053 4.80	0.270 0.054 4.97	0.289 0.053 4.80
Constant		0.292 0.090 3.23	0.291 0.092 3.18	0.292 0.090 3.23
Adjusted R^2 std error		0.487 0.046	0.494 0.045	0.494 0.044

a:$\log_{10}$

The final analysis was repeated omitting parental values and protein intake, thus including only the biological variables age, sex, pubertal status, height, weight and skinfold thickness, each of which is easily determined. The details of these regression analyses are shown in Table 7.3. In each set the variance accounted for by the regression decreased 3 to 7% and the standard error increased slightly. Age was no longer a significant predictor of BW.

7.2 RATE OF CHANGE IN BONE STATUS

A similar analysis was performed for the two year change in each bone variable. The independent variables were sex, initial (I) age, initial (I) and final (F) pubertal status as dummy variables PHS2 to PHS5, height, weight and skinfold thickness initial values and velocities, calcium, energy and protein intakes as means of the initial and final intakes, physical activity score as mean of the initial and final scores, the respective initial bone variable and mother's and father's respective bone variables. The results are presented in Table 7.4. 52% of the variance of the change in BMC was accounted for, 55% of the change in BW, 47% of the change in aBMD and 58% of the change in vBMD. Sex had a negative effect on the change in BMC indicating that velocity was greater in boys than in girls. Sex and initial BW had a negative effect on BW velocity indicating a lower velocity in girls and a lower velocity in those with greater BW. Similarly, sex and initial aBMD had a negative effect on aBMD velocity. Sex was not a significant predictor of vBMD velocity and height velocity and initial vBMD had a negative effect, ie the greater the height velocity the smaller the vBMD velocity and the greater the initial vBMD the smaller the vBMD velocity.

Pubertal status was a significant contributing factor to the variance of each bone variable except BW. Mother's bone variables were significant positive predictors of BW and aBMD velocities only, while father's bone variables were not significant for any bone variable, nor were nutrient intake variables or physical activity score. To confirm that these variables and others not included in individual equations (eg PHS in BW) were not significant predictors

Table 7.3
Details of multiple regression analysis for each bone variable, omitting parental and nutrient variables

BMC (g/cm) Adjusted $R^2 = 0.767$, standard error = 0.145

Variable	Sex	PHS4	PHS5	Age	Weight	Total skinfold[a]	Constant
Units	male = 0 female = 1	PHS 4 = 1 else= 0	PHS 5 = 1 else = 0	years	kg	mm	
B	-0.056	0.058	0.108	0.027	0.013	-0.530	0.727
se B	0.018	0.024	0.030	0.007	0.001	0.058	0.113
t	3.12	2.42	3.66	4.12	13.06	9.16	6.41

a: $\log_{10}$

BW (cm) Adjusted $R^2 = 0.638$, standard error = 0.224

Variable	Sex	PHS3	Height	Weight	Constant
Units	male = 0 female = 1	PHS 3 = 1 else = 0	cm	kg	
B	-0.202	0.088	0.013	0.0077	0.325
se B	0.023	0.029	0.0014	0.0012	0.183
t	8,94	2.99	9.35	6.47	1.77

Table 7.3 continued

aBMD (g/cm^2) Adjusted R^2 =0.720, standard error = 0.037

Variable	PHS4	PHS5	Age	Height	Weight	Total skinfold[a]	Constant
Units	PHS 4 = 1 else = 0	PHS 5 = 1 else = 0	years to 0.1	cm	kg	mm	
B	0.030	0.048	0.011	-0.0014	0.0026	-0.125	0.450
se B	0.006	0.008	0.002	0.0004	0.0003	0.015	0.058
t	4.86	6.19	6.18	3.77	7.88	8.12	7.73

a: $\log_{10}$

vBMD (g/cm^3) Adjusted R^2 = 0.453, standard error = 0.047

Variable	Sex	PHS2	PHS3	Age	Height	Weight	Total skinfold[a]	Constant
Units	male = 0 female = 1	PHS 2 = 1 else= 0	PHS 3 = 1 else= 0	years	cm	kg	mm	
B	15.3	-25.6	-41.0	17.9	-2.27	1.56	-90.0	557
se B	5.77	7.18	6.48	1.93	0.44	0.45	22.5	72.6
t	2.63	3.57	6.32	9.26	5.12	3.49	4.00	7.67

a: $\log_{10}$

Table 7.4
Details of multiple regression analysis for change in each bone variable

Change in BMC (g/cm/2yr) Adjusted $R^2 = 0.519$, standard error = 0.081

Variable	Sex	PHS2 I	PHS3 I	PHS4 I	PHS5 I	Initial height	Change in height	Constant
Units	male = 0 female=1	PHS 2 = 1 else = 0	PHS 3 = 1 else = 0	PHS 4 = 1 else = 0	PHS 5 = 1 else = 0	cm	cm/2 years	
B	-0.021	0.045	0.124	0.085	0.063	0.005	0.013	-0.736
se B	0.011	0.015	0.017	0.021	0.024	0.0006	0.001	0.099
t	1.91	3.03	7.24	4.11	2.58	7.58	10.58	7.40

Change in BW (cm/2 yr) Adjusted $R^2 = 0.545$, standard error = 0.121

Variable	Sex	Change in height	Initial BW	Mother's BW	Constant
Units	male = 0 female = 1	cm/2 years	cm	cm	
B	-0.90	0.017	-0.119	0.079	0.187
se B	0.018	0.0015	0.027	0.032	0.097
t	5.08	10.91	4.44	2.46	1.92

Table 7.4 continued

Change in aBMD (g/cm^2/2 yr) Adjusted $R^2 = 0.471$, standard error = 0.028

Variable	PHS2 I	PHS3 I	PHS4 I	PHS4 F	PHS5 F	Sex	Initial height	Initial aBMD	Constant
Units	PHS 2= 1 else = 0	PHS 3= 1 else = 0	PHS 4= 1 else = 0	PHS 4= 1 else = 0	PHS 5= 1 else = 0	male = 0 female=1	cm	g/cm^2	
B	0.013	0.029	0.014	0.021	0.021	-0.0065	0.0016	-0.289	-0.139
se B	0.0048	0.0051	0.0053	0.0050	0.0059	0.0034	0.0002	0.0406	0.030
t	2.60	5.76	2.73	4.07	3.50	1.91	7.52	7.12	4.63

Change in vBMD (g/cm^3/2 yr) Adjusted $R^2 = 0.578$, standard error = 0.034

Variable	PHS2 I	PHS3 I	PHS4 I	PHS5 I	PHS4 F	PHS5 F	Initial height	Change in height	Initial vBMD	Mother's vBMD	Constant
Units	PHS 2= 1 else = 0	PHS 3= 1 else = 0	PHS 4= 1 else = 0	PHS 5= 1 else = 0	PHS 4= 1 else = 0	PHS 5= 1 else = 0	cm	cm/2 years	g/cm^3	g/cm^3	
B	15.6	36.6	39.1	24.9	25.6	20.0	1.10	-1.98	-0.52	0.11	-29.6
se B	6.6	8.5	10.8	12.6	6.5	8.5	0.28	0.54	0.048	0.041	50.2
t	2.37	4.33	3.64	1.97	3.95	2.36	3.92	3.64	10.64	2.77	0.59

each was forced into the equation. The new equations together with the adjusted R^2 are shown in Table 7.5.

Physical activity was the only variable that was a significant predictor of BMC velocity when forced into the equation. The effect of physical activity was negative ie the greater the physical activity score the smaller the change in BMC. Addition of this variable increased R^2 by 1.1%, increased the coefficient for sex and decreased the coefficients for PHS4F and PHS5F. While there was little change in the significance of sex and PHS4F, PHS5F changed from being significant at the 1% level to being non-significant at 5%. This indicates a strong correlation between PHS5F and physical activity and that there is a substitutability between the two. Addition of father's BMC increased R^2 by 5% but this variable was not significant.

Addition of non-significant variables to the equation for BW velocity confirmed that these variables including calcium intake and physical activity were not significant predictors. All increases in R^2 were less than 0.5%. None of the variables forced into the equation was a significant predictor of aBMD. The greatest change in R^2 was observed when father's aBMD was forced in and that was 4.8%. Small changes occurred in all coefficients but the significance of the variables did not change. Addition of non-significant variables to the equation for vBMD velocity resulted in small increases in R^2 (less than 3%), and confirmed that these variables, including calcium intake, physical activity, sex and father's vBMD, were not significant predictors of vBMD velocity.

Table 7.5
Details of multiple regression analysis for change in each bone variable and new equations resulting from forced entry of each additional variable
For each variable: B (top line), se B (second line), t (third line)

Change in BMC (g/cm/2yr)

Variable	Units	Original equation	Forced entry variables						
			Calcium g/day	Physical activity score	Mother's BMC g/cm	Father's BMC g/cm	Initial age years	Final age	Initial BMC g/cm
			0.00002	-0.004	0.029	0.020	0.002	0.002	0.047
			0.00001	0.001	0.030	0.023	0.006	0.006	0.034
			1.38	2.98	0.97	0.86	0.39	0.35	1.36
Sex	male=0	-0.027	-0.014	-0.030	-0.028	-0.036	-0.026	-0.026	-0.019
	female=1	0.011	0.012	0.011	0.011	0.011	0.11	0.011	0.012
		2.50	1.15	2.74	2.57	3.21	2.34	2.35	1.61
PHS3I	phs 3 = 1	0.074	0.076	0.074	0.073	0.083	0.073	0.074	0.075
	else=0	0.013	0.013	0.013	0.013	0.014	0.013	0.013	0.013
		5.82	5.84	5.69	5.65	6.03	5.72	5.73	5.87
PHS4I	phs 4 = 1	0.035	0.033	0.035	0.043	0.044	0.036	0.036	0.036
	else = 0	0.014	0.015	0.015	0.014	0.015	0.014	0.015	0.014
		2.50	2.21	2.37	2.95	2.88	2.50	2.50	2.56
PHS3F	phs 3 = 1	0.042	0.043	0.040	0.044	0.037	0.041	0.041	0.041
	else =0	0.017	0.017	0.017	0.018	0.019	0.017	0.017	0.017
		2.43	2.48	2.36	2.52	1.98	2.36	2.36	2.38
PHS4F	phs 4 = 1	0.063	0.062	0.059	0.068	0.065	0.061	0.061	0.061
	else = 0	0.019	0.019	0.019	0.020	0.020	0.020	0.020	0.019
		3.30	3.28	3.12	3.45	3.19	3.09	3.10	3.20
PHS5F	phs 5 = 1	0.052	0.051	0.040	0.062	0.051	0.048	0.048	0.048
	else = 0	0.021	0.021	0.021	0.022	0.022	0.023	0.023	0.021
		2.46	2.41	1.86	2.88	2.27	2.07	2.08	2.27
Initial height	cm	0.003	0.003	0.003	0.003	0.004	0.003	0.003	0.003
		0.0007	0.0007	0.0007	0.0007	0.0008	0.0008	0.0008	0.0008
		4.59	4.18	4.48	4.48	4.57	3.92	3.94	4.05
Change in height	cm	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.014
		0.001	0.001	0.001	0.001	0.001	0.0008	0.001	0.001
		11.30	11.25	11.49	10.74	10.50	10.58	10.55	10.90
Initial weight	kg	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006
		3.67	3.95	3.68	2.72	2.59	3.68	3.68	3.31
Constant		-0.609	-0.611	-0.575	-0.634	-0.669	-0.617	-0.621	-0.606
		0.102	0.104	0.104	0.105	0.118	0.105	0.108	0.102
		5.96	5.89	5.53	6.03	5.66	5.89	5.76	5.94
Adj R^2		0.545	0.545	0.556	0.552	0.596	0.544	0.544	0.546
std error		0.079	0.078	0.077	0.078	0.073	0.079	0.079	0.078

Table 7.5 continued

Change in BW (cm/2 yr)

Variable	Units	Original equation	Forced entry variable								
			Calcium g/day	Physical activity score	PHS2I	PHS3I	PHS4I	PHS5I	Father's BW cm	Initial age years to 0.1	Final age
			3.2×10^{-5}	-0.001	0.002	-0.022	-0.001	0.042	0.002	0.008	0.009
			2.1×10^{-5}	0.002	0.019	0.018	0.019	0.025	0.003	0.007	0.007
			1.52	0.53	0.12	1.22	0.06	1.64	0.66	1.23	1.35
Sex	male=0 female=1	-0.090	-0.074	-0.090	-0.090	-0.087	-0.090	-0.087	-0.105	-0.087	-0.087
		0.018	0.020	0.019	0.018	0.018	0.018	0.018	0.020	0.018	0.018
		5.08	3.78	4.67	5.07	4.92	5.07	4.92	5.15	4.94	4.92
Change in height	cm/2yr	0.017	0.016	0.017	0.017	0.017	0.017	0.018	0.016	0.018	0.018
		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
		10.91	10.40	10.40	10.39	10.99	10.28	10.86	9.02	9.89	9.95
Initial BW	cm	-0.119	-0.127	-0.123	-0.118	-0.109	-0.119	-0.123	-0.144	-0.137	-0.138
		0.027	0.028	0.028	0.027	0.028	0.027	0.027	0.027	0.030	0.030
		4.44	4.62	4.40	4.40	3.92	4.41	4.59	4.61	4.49	4.55
Mother's BW	cm	0.072	0.076	0.086	0.079	0.071	0.079	0.081	0.100	0.088	0.088
		0.032	0.034	0.035	0.032	0.033	0.032	0.032	0.038	0.033	0.033
		2.46	2.20	2.45	2.44	2.16	2.45	2.50	2.63	2.66	2.68
Constant		0.187	0.182	0.187	0.186	0.185	0.187	0.181	0.148	0.089	0.062
		0.097	0.102	0.108	0.097	0.097	0.097	0.097	0.143	0.125	0.134
		1.92	1.78	1.73	1.91	1.91	1.92	1.87	1.04	0.71	0.46
Adj R^2		0.545	0.532	0.531	0.544	0.546	0.544	0.548	0.548	0.546	0.547
std error		0.121	0.121	0.123	0.121	0.121	0.121	0.120	0.122	0.121	0.121

Table 7.5 continued

Change in aBMD (g/cm^2/2 yr)

Variable	Units	Original equation	Forced entry variable						
			Calcium g/day	Physical activity score	Father's aBMD g/cm^2	Mother's aBMD g/cm^2	Change in height cm/2yr	Initial age years	Final age
			8.0×10^{-7} 4.7×10^{-6} 0.17	-0.001 0.0005 1.86	0.037 0.035 1.06	0.071 0.038 1.88	0.0007 0.0005 1.57	0.0006 0.002 0.32	0.0004 0.002 0.20
Sex	male=0 female=1	-0.007 0.003 1.91	-0.006 0.004 1.37	-0.008 0.004 2.20	-0.011 0.004 2.89	-0.007 0.004 2.04	-0.003 0.004 0.72	-0.007 0.003 1.90	-0.007 0.003 1.90
PHS2I	PHS 2=1 else=0	0.013 0.005 2.60	0.012 0.005 2.43	0.011 0.005 2.32	0.014 0.005 2.60	0.012 0.005 2.31	0.011 0.005 2.36	0.013 0.005 2.60	0.013 0.005 2.60
PHS3I	PHS 3=1 else=0	0.029 0.005 5.76	0.030 0.005 5.61	0.029 0.005 5.57	0.031 0.006 5.43	0.029 0.005 5.52	0.029 0.005 5.79	0.029 0.005 5.64	0.029 0.005 5.67
PHS4I	PHS 4=1 else=0	0.014 0.005 2.73	0.015 0.006 2.66	0.015 0.006 2.62	0.020 0.006 3.34	0.017 0.005 3.10	0.014 0.005 2.85	0.014 0.005 2.72	0.014 0.005 2.72
PHS4F	PHS 4=1 else=0	0.021 0.005 4.07	0.021 0.005 4.13	0.021 0.005 4.02	0.021 0.005 3.88	0.022 0.005 4.16	0.020 0.005 3.86	0.021 0.005 3.95	0.020 0.005 3.97
PHS5F	PHS 5=1 else=0	0.021 0.006 3.50	0.021 0.006 3.49	0.018 0.006 2.93	0.020 0.007 3.07	0.023 0.006 3.80	0.020 0.006 3.42	0.021 0.006 3.13	0.020 0.006 3.18
Initial aBMD	g/cm^2	-0.289 0.041 7.12	-0.300 0.042 7.11	-0.296 0.042 6.98	-0.357 0.046 7.74	-0.330 0.041 7.82	-0.252 0.047 5.36	-0.293 0.043 6.88	-0.292 0.043 6.84
Initial height	cm	0.002 0.0002 7.52	0.002 0.0002 7.25	0.002 0.0002 7.29	0.002 0.0003 7.02	0.002 0.0002 7.01	0.002 0.0002 7.63	0.002 0.0002 6.38	0.002 0.0002 6.44
Constant		-0.139 0.030 4.63	-0.136 0.031 4.37	-0.127 0.031 4.05	-0.156 0.037 4.17	-0.148 0.033 4.44	-0.173 0.038 4.61	-0.140 0.030 4.64	-0.141 0.031 4.58
Adj R^2 std error		0.471 0.028	0.474 0.028	0.480 0.028	0.519 0.027	0.476 0.028	0.470 0.028	0.469 0.028	0.469 0.028

Table 7.5 continued
Change in vBMD (g/cm^3/2yr)

Variable	Units	Original equation	Forced entry variable					
			Calcium g/day	Physical activity score	Sex	Initial age	Final age	Father's vBMD g/cm^3
			$-6.0x10^{-6}$	-0.0009	0.007	0.0025	0.002	0.059
			$5.3x10^{-6}$	0.0006	0.005	0.0026	0.003	0.043
			1.15	1.40	1.36	0.99	0.87	1.38
PHS2I	PHS 2=1 else = 0	0.016	0.014	0.012	0.016	0.015	0.015	0.018
		0.007	0.007	0.006	0.007	0.007	0.007	0.007
		2.37	2.08	1.92	2.41	2.28	2.29	2.45
PHS3I	PHS 3=1 else=0	0.037	0.036	0.035	0.038	0.034	0.034	0.038
		0.008	0.008	0.008	0.008	0.009	0.009	0.009
		4.33	4.25	4.15	4.44	3.86	3.90	4.10
PHS4I	PHS 4=1 elese=0	0.039	0.042	0.040	0.040	0.037	0.037	0.046
		0.011	0.011	0.011	0.011	0.011	0.011	0.012
		3.64	3.88	3.72	3.68	3.37	3.34	4.01
PHS5I	PHS 5=1 else=0	0.025	0.025	0.024	0.026	0.022	0.022	0.031
		0.013	0.013	0.013	0.013	0.013	0.013	0.014
		1.97	1.90	1.87	2.08	1.65	1.68	2.29
PHS4F	PHS 4=1 else=0	0.026	0.027	0.029	0.023	0.025	0.025	0.024
		0.006	0.006	0.006	0.007	0.006	0.006	0.007
		3.95	4.19	4.02	3.54	3.84	3.86	3.46
PHS5F	PHS 5=1 else=0	0.020	0.022	0.019	0.018	0.018	0.018	0.015
		0.008	0.008	0.009	0.008	0.009	0.009	0.009
		2.36	2.55	2.22	2.16	2.09	2.12	1.59
Initial height	cm	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003
		3.92	3.99	4.05	4.15	2.99	3.05	3.97
Change in height	cm/2yr	-0.002	-0.002	-0.002	-0.002	-0.002	-0.002	-0.0016
		0.0005	0.0005	0.0005	0.0006	0.0005	0.0005	0.0006
		3.64	2.99	3.18	2.52	3.53	3.54	2.67
Initial vBMD	g/cm^3	-0.516	-0.535	-0.548	-0.501	-0.523	-0.523	-0.533
		0.048	0.049	0.050	0.050	0.049	0.049	0.053
		10.64	10.86	10.94	10.13	10.66	10.63	10.04
Mother's vBMD	g/cm^3	0.114	0.117	0.128	0.115	0.117	0.116	0.127
		0.041	0.043	0.042	0.041	0.041	0.041	0.047
		2.77	2.73	3.01	2.78	2.82	2.81	2.68
Constant		-0.030	-0.030	-0.027	-0.060	-0.036	-0.040	-0.088
		0.050	0.050	0.050	0.055	0.051	0.052	0.058
		0.59	0.58	0.54	1.09	0.72	0.77	1.51
Adj R^2		0.579	0.597	0.607	0.580	0.579	0.578	0.606
std error		0.034	0.034	0.034	0.034	0.034	0.034	0.034

DISCUSSION

7.1 Absolute bone status

Multivariate analysis was used to determine the significant factors which described the variance of bone size, mineral content and density. Several authors have performed similar analyses in children and adolescents (Sentipal et al 1991, Katzman et al 1991, Thomas et al 1991, Southard et al 1991, Grimston et al 1992, Lloyd et al 1992, Miller et al 1991, Gunnes and Lehmann 1995). Many of these have considered only age and variables relating to body size, and none has included such a wide range of variables encompassing both environmental and genetic factors as well as the biological factors of body size and pubertal status.

The study by Gunnes and Lehmann (1995) of 321 adolescents aged 11to 17 years reported that 57% of the variance of aBMD of the distal radius was accounted for by, age, height, weight and fat intake. Inclusion of pubertal status may in part explain the greater variance (72%) that was accounted for in the present study. Addition of pubertal status variables to the equation after age, height, weight and skinfold thickness, increased R^2 by 5%. Only 2% of the variance was explained by fat intake and this may have reflected gender differences similar to protein intake in the present study. Calcium intake and physical activity were not significant predictors, a similar finding to the present study.

Southard et al (1991) reported that 85% of the variance of vertebral aBMD was explained by weight and Tanner stage in 218, 1 to 19-year-olds. They found that age, sex, diet and activity added little to the prediction equation. Similarly Grimston et al (1992) reported that pubertal stage or body weight accounted for 77% or 68% respectively of the variability in spinal aBMD of 74, 9 to 16-year-olds and suggested that the differences in aBMD in this age group occur mainly as a function of puberty and the associated increases in body weight.

The study by Miller et al (1991) was on younger subjects aged 5 to 14 years and included only age and a range of anthropometric variables. Analyses were performed separately on boys and girls. For the distal wrist they reported that 80% and 86% of BMC and 71% and 68% aBMD

in boys and girls respectively was accounted for by height and up to three other measures such as skinfold and arm circumference. They reported similar values at other skeletal sites. Sentipal et al (1991) also measured vertebral aBMD in 49 females aged 8 to 18 years and reported that 81% of the variance was accounted for by age, pubertal stage and calcium intake. Energy expenditure calculated from a 3-day activity diary was not a significant predictor.

The results of Katzman et al (1991) most closely resemble this study. They measured BMC, aBMD and vBMD at a number of skeletal sites in 45 girls aged 9 to 21 years. In the radius, 77% of the variance of BMC, 79% of aBMD and 35% of vBMD, were accounted for by age, Tanner score, height and weight, although not all these variables were significant predictors. The lower variance of vBMD as compared with BMC and aBMD was also seen at other sites and parallels the finding of the present study.

The only study to have included genetic factors in multiple regression analysis was a study by Henderson et al (1995) in 118, 18-year-old women. They measured aBMD at a number of sites and related this to nutrient intake, aerobic fitness, trunk muscle strength, habitual activity level and body weight. Depending on the site, between 13 and 27% of the variance of aBMD was predicted by weight, weight-corrected flexor strength, and weight-corrected estimated oxygen uptake. When mother's aBMD was included in the analysis the prediction increased to between 18 and 31% depending on the site. Nutrient intake and habitual activity level were not significant predictors. This increase of 4 to 5% in the predicted variance by inclusion of maternal aBMD is similar to the 3 to 7% increase observed in the present study by addition of both maternal and paternal bone variables.

The positive association of protein intake with bone mass in this study is in contrast to the expected negative effect of protein due to its role in increasing urinary calcium (Heaney and Recker 1982), and the results of two studies in young adult women (Metz et al 1993, Recker et al 1992). Metz reported that protein intake had a negative effect on radial BMC and aBMD and Recker showed that the rates of gain in spinal BMC and aBMD were positively related to the calcium/protein ratio of the diet. The inclusion of protein intake in multiple regression

equations in the present study made little or no difference to the overall variance accounted for by the equation. Protein intake in this instance probably reflects gender differences in BMC, BW and aBMD.

In the present study of subjects aged 11 to 17 years multiple regression analysis has enabled the formulation of prediction equations which account for 72 to 80% of the variance of BMC, BW and aBMD. The lower value of 49% of the variance of vBMD accounted for does not enable this variable to be usefully predicted. When parental variables are removed from the equation, the variance accounted for by the prediction equations is reduced remains at a level high enough for these equations to be useful in respect of BMC, BW and aBMD.

7.2 Rate of change in bone status

Only two published studies have determined the factors contributing to change in radial bone mass in children (Goslings et al 1995, Gunnes and Lehmann 1996). The study by Goslings was on 392 children aged 6 to 12 years who had been seen two years earlier. They reported that 21% of the variance of BW velocity was predicted by weight and height velocity, and the mean of the initial and final BW. 49% of the variance of BMC velocity was predicted by BW velocity, height and weight velocities, and the mean of the initial and final height and BMC. The present study also found that initial BW and height velocity were significant predictors of BW velocity and initial height and height velocity were significant predictors of BMC velocity.

Gunnes and Lehmann made two measurements, one-year apart, of forearm aBMD in 470 boys and girls aged 8 to 16 years (Gunnes and Lehmann 1996). In girls aged 11 years and over, initial height and aBMD explained 19% of the variance of aBMD velocity, and in boys initial height and weight-bearing physical activity explained 26% of the variance of distal forearm aBMD. Pubertal status was not determined in either study.

Overall a greater percent of the variance of all bone variables was accounted for in the present study, particularly compared with the results of Gunnes and Lehmann (1996). The inclusion of variables of pubertal status may explain some of these differences. The multiple regression

results of the present study further demonstrate the strong influence on bone mass accrual (BMC, aBMD, vBMD) of pubertal changes in the adolescent years. In BW this influence is exerted through changes in height.

The negative association of the initial value of the bone variable with velocity of BW, aBMD and vBMD indicates that velocity decreases as values approach adult levels. This observation has implications with respect to optimising bone accrual through modifiable factors. Velocities were greatest in the mid to late pubertal years when subjects were progressing from pubertal stage 3 to pubertal stages 4 and 5. The ability to increase bone density through modifiable factors may be greater when bone gain is faster, ie in the mid pubertal years, than in the late stages of puberty when bone density velocity is much less. However it may be that the rapid bone gain in mid-puberty under hormonal influence does not allow the much smaller influences of environmental factors to be expressed.

In summary the results presented in this chapter support the hypothesis that the significant indicators of forearm bone mineral content, width and density in boys and girls aged 11 to 17 years include age, gender, sexual maturity, variables of body size and father's and mother's respective bone variables. The results reject the hypothesis that the environmental factors of calcium intake and physical activity are significant predictors. These results also support the hypothesis that the significant predictors of the velocities of bone mineral content, width and density, are gender, sexual maturity and variables of body size. For BW and vBMD velocities, the results support the hypothesis that the respective maternal bone variable is a significant predictor. The hypothesis that age, father's respective bone variable, calcium intake and physical activity are significant predictors of bone variable velocity is rejected for all bone variables, and similarly that mother's respective bone variable is a significant predictor of BMC and aBMD velocities is also rejected. However the demonstration in chapter 5 that girls with a consistently low calcium intake had a significantly lower rate of increase in BMC, aBMD and vBMD than girls with a consistently high intake suggests that adequate nutrition is an important factor in bone status in the adolescent years.

CHAPTER 8

FINAL DISCUSSION

Study design and outcome

An observational study design was selected at the commencement of the study as there were almost no data available on the pattern of changes in bone growth through puberty, nor any data in an Australian paediatric population. The bone mass and density results from the study have provided normal data for age and for pubertal status. These data are now used by the Department of Nuclear Medicine, Royal Adelaide Hospital, to provide the reference ranges for osteoporosis evaluation of adolescents.

Specifically, the study has documented the bone status and rate of change in bone status in a group of normal Australian boys and girls as they progress through puberty. Data collection spanned six years with measurements made four times at two year intervals. Most of the subjects had been enrolled in a larger longitudinal study initiated at birth, and nutrient intake data were available from the age of four years. The study therefore spans a longer period than any published studies of bone status in adolescents. It includes measures of forearm bone mass, bone width and bone density. It has shown that bone status was dependent on age, gender and sexual maturity but that gender differences were greater for bone mineral content and bone width than for bone mineral density. Bone mass and density were near their peak (97%) in girls at skeletal maturity, as judged by epiphyseal closure, but in boys were up to 10% below their peak at skeletal maturity. Cross-sectional bone status was not related to nutrient intake or physical activity but was significantly associated with parental bone status, particularly maternal bone status.

Longitudinal nature of the study

An important feature of this study is its longitudinal nature. The advantage of a longitudinal study is that it eliminates inter-subject variation and provides true estimates of rates of change in individuals that can be related to measures of maturation such as menarche and the cessation

of longitudinal growth. A potential disadvantage of longitudinal studies is subject attrition. Only five subjects (<5%) were lost from the study between the first and third measurements, and a further nine declined to participate in the final measurement at 17 years. In addition, fourteen subjects did not complete the diet and activity diaries. The overall completion rate for the study was 86% for the bone measurements and 74% for the diet and activity diaries. This decline in participation and failure to complete fully the study requirements are the consequences of unpredictable changing family circumstances and a decline in subject interest as they mature and become able to make their own decisions. It illustrates the difficulties encountered in studying adolescents who in their older years may be in employment, have busy lifestyles or who see participation as an infringement on their freedom despite the wish of their parents. The completion of a food intake diary was seen as particularly onerous and invasive by many. However there is no reason to believe the intakes of those subjects who failed to keep a record differed from those of others in the study.

Changes in bone size and effect on bone mineral density

Another problem in assessing bone status of growing subjects is the effect of changing bone size. Most studies in children and adolescents have reported bone density as an areal bone mineral density which does not account for the third dimension, and so does not fully reflect the actual bone density. This error is partly offset by a reduction of the inter-individual variation in a longitudinal study compared with a cross-sectional study. The use of volumetric bone density also reduces the error. In the present study derivation of the cross-sectional area of the bone allowed estimation of volumetric bone mineral density. The observation, previously unreported, of a fall in forearm volumetric bone density in boys at the time of rapid longitudinal growth stems from this calculation and may be a factor associated with the relatively high fracture rates in boys at this age.

Yet another problem with sequential bone measurements during growth is the difficulty in repositioning. The forearm site selected for scanning was the point at which the bones were 8 mm apart. Is this region the same at age 17 as it was at age 11? There is no definitive answer to this question.

Age of attainment of peak bone status

The unique contribution of this study is the determination of the timing of attainment of peak bone status in relation to longitudinal cessation of growth, in boys and girls. The rate of change in bone status in girls between 15 and 17 years and comparison of values at age 17 years with the mean adult premenopausal value suggest that increases in subsequent years are likely to be small. Bone values at 17 years in the skeletally mature girls, as assessed by radial epiphyseal fusion, showed them to be near peak bone mass (103%) and density (97%). However further measurements are needed to determine precisely the age at which peak bone mass and density are attained. This can only be determined when bone status has stabilised. As yearly increments are likely to be small, repeated measures for additional periods of up to five to ten years may be required.

In contrast, the rate of change in bone status in boys between 15 and 17 years and comparison of values at age 17 with adult male values suggest that significant increases in bone mass and density are likely to occur beyond the age of 17 years. These observations, and the rates of change in bone status in those boys skeletally mature at 17 years, suggest that boys are further from peak bone mass and density than girls at the same age and that bone accrual continues beyond skeletal maturity to a greater extent in boys than in girls. However precise determination of the age of attainment of peak bone mass and density in boys also requires measurements for a further five to ten years..

Effect of environmental factors on bone status in adolescence

The high level of tracking of bone status observed in this study suggests that there is limited scope for modification of bone mass and density by environmental factors in this adolescent period. However any gain in bone mass or density is advantageous in reducing any later risk of osteoporosis. The nutrient intakes and physical activity levels of subjects in this study displayed a wide range of values at each age and showed considerable variability from one age to the next. This observation, and the methodological difficulties in the precise determination of nutrient intake and activity level, may explain the lack of significant correlation between these measures and bone status and rate of change in bone status. Currently available methods

for assessing physical activity as it relates to mechanical stress on the skeleton overall, and on skeletal components are far from ideal. This is an important area for further study. If the response of growing bone to specific activities can be quantified precisely the relative effects on peak bone status of participation in high response and low response activities could be determined. Identification of a positive effect of specific activities would enable development of more precise guidelines with respect to exercise participation for maximising peak bone status.

Few subjects had physical activity scores or nutrient intakes consistently at the extremes because of individual variation in these factors between measuring points. This leads to the possibility of a type II error. However the significantly greater increase in bone mass and density in the six-year study period in girls on consistently high calcium intakes in comparison with those on consistently low intakes suggests that bone status during growth is compromised by a low calcium intake. This finding requires further investigation in a larger group of subjects from late childhood/early adolescence, identified by their low calcium intake. Individuals with lactose or cow's milk protein intolerance or a dislike of dairy products are obvious examples. The hypothesis that a consistently low calcium intake (<0.7RDI) during adolescence may result in a reduced peak bone mass and density may be tested by further study. This should be a prospective study of female subjects initially aged 10 to 11 years with known low calcium intake randomised to a treatment (calcium supplementation preferably with dairy products equivalent to 500 or 1000 mg per day) or control group, and monitored for ten years until peak bone mass and density are reached. Measurements needed would include estimation of bone mass and volumetric density and matching of subjects for their level of physical activity.

Identification of a target population

Whilst both mothers' and fathers' bone variables were significant predictors of the respective children's bone variables, correlations were stronger between mothers and their children than between fathers and their children. The reasons for these gender differences are not clear. However this finding may be important in the prevention of osteoporosis by indicating a

potential target group of children who are likely to be at greater risk of a low bone mass because of known osteoporosis in mother or grandmother.

Osteoporosis is an increasing health care problem in our ageing society. The aim of current treatment is to halt further bone loss as it is not possible to reverse previous loss to any substantial degree. As the risk of osteoporotic fracture is related to the bone density at that site, which in turn is related to the peak bone density attained in early adulthood, maximising peak bone mass and density is an important long-term community strategy for prevention of osteoporosis. Therefore identification of a target population for intervention is a significant contribution to any strategic plan for prevention.

Summary

The results of this study indicate that the years of rapid bone growth for girls are from the age of 8 or 9 to 17 years. It is slightly later in boys consistent with their later sexual development. While this study has not shown any associations of bone status with variations in physical activity and little variance with respect to calcium intake, mechanical stress, achieved through physical activity, and calcium are important factors for maintaining bone health throughout life. Further studies are required in order to identify more precisely what are the appropriate lifestyle behaviours that will maximise bone status. However assignment of subjects to a control group, for example a group with calcium intakes consistently below 70% of the recommended dietary intake, does raise some ethical issues.

Meanwhile the public health messages of the benefits of good nutrition and regular physical activity should be targetted at children before and during their period of rapid growth so that their genetic potential of peak bone status can be achieved. This particularly applies to girls who have overall lower nutrient intakes and lower levels of physical activity than boys and to daughters of osteoporotic mothers and grandmothers. The social pressure on girls to be thin and the consequent incidence of inappropriate eating patterns in the early adolescent years leads to low calcium intakes. Such a group is an important target population for education and

lifestyle changes. Childhood and adolescence are important times to establish appropriate patterns of behaviour that can be maintained in adult life.

The difficulty of attempts to change the lifestyle of children and adolescents in order to reduce the risk of later osteoporosis is that the consequences of sub-maximal peak bone mass are not seen for two or three decades. Osteoporosis is one of a number of diseases which are a consequence of our changed nutrition and sedentary lifestyle compared with our early ancestors (Eaton 1992). The proposed improvements in nutrition and physical activity will be beneficial with respect to other diseases of the Western world, particularly heart disease and high blood pressure. Although dairy products are the best source of dietary calcium they can be high in fat, particularly saturated fat. Therefore population based nutritional instruction in youth needs to highlight selection of dairy products which are low in fat. This will ensure that attempts to improve the nutritional environment for prevention of one disease (osteoporosis) do not increase the risk of another (ischaemic heart disease).

APPENDIX

Publications from the Adelaide Nutrition Study

Magarey A, Boulton TJC. 1984. Nutritional studies during childhood: IV Energy and nutrient intake at age 4. Aust Paed J 20:187-194.

Magarey A, Boulton TJC. 1987. Children's thinking about food. 1. Knowledge of nutrients. J Food and Nutrition 43:2-9.

Magarey A, Boulton TJC. 1987. Children's thinking about food. 2. Concept developments and beliefs. J Food and Nutrition 43:9-16.

Magarey A, Boulton TJC. 1987. Percentiles of food energy, macro and micronutrient intake from infancy to age 8 years. Med J Aust 147:124-126.

Boulton TJC, Magarey A. 1987. Precursors of cardiovascular risk factors in childhood. A family study. In Cardiovascular Risk Factors in Childhood. Eds Hetzel B, Berenson GS. Elsevier. The Hague Science Publishers (Biomedical Division) 43-65.

Magarey A, Boulton TJC. 1987. Energy and nutrient intake at age 6 and its relationship to body size and fatness. Aust Paed J 23:41-46.

Magarey A, Boulton TJC. 1987. Food intake at age 8. 1 Energy, macro and micronutrient intake. Aust Paed J 23:173-178.

Magarey A, Boulton TJC. 1987. Food intake at age 8. 2 Company, place and time of eating. Aust Paed J 23:179-180.

Magarey A, Boulton TJC. 1987. Food intake at age 8. 3 Nutrient intake by meal. Aust Paed J 23:217-221.

Boulton TJC, Magarey A, Nichols J. 1987. Food patterns in youth: their effect on later life. In Food and Health Issues and Directions. Eds Wahlqvist M, King R, McNeil JJ, Sewell R. J Libbey UK. 6-11.

Boulton TJC, Magarey A. 1988. Cholesterol and obesity in childhood-implications for future health. Aust J Earl Childhood 13:10-19.

Boulton TJC, Magarey A, Cockington R. 1990. Cholesterol from infancy to age thirteen: tracking and parent-child associations. Annales Nestle 48:70-76.

Magarey A, Seal J, Boulton TJC. 1991. Calcium, fat and P/S ratio in children's diets: a mixed public health message. Aust J Nut Diet 48:58-61.

Boulton TJC, Seal J, Magarey A. 1991. Cholesterol in childhood: how high is OK? Recommendations for screening, case finding and intervention. Med J Aust 154:847-850.

Magarey A, Boulton TJC. 1991. A comparison of British and Australian nutrient composition tables for the estimation of fat in the diets of 11-year-old children. Aust J Nutr Diet 48:128-131.

Magarey AM, Daniels LA, Boulton TJC. 1993. Reducing the fat content of children's diets; nutritional implications and practical recommendations. Aust J Nutr Diet 50:67-72.

Magarey AM, Boulton TJC, Daniels LA, Davidson GP. 1994. Recommendations for dietary intervention in the prevention and treatment of hyperlipidaemia in childhood: a consensus statement from the Dietitians Association of Australia and the Australian College of Paediatrics. Aust J Nutr Dietet.51:191-198.

Magarey AM, Boulton TJC. 1994. The Adelaide Nutrition Study 1. Food energy through adolescence: including an evaluation of the pattern f under-recording, age and sex differences. Aust J Nutr Dietet 51:104-110.

Magarey AM, Boulton TJC. 1994. The Adelaide Nutrition Study 2. Macronutrient and micronutrient intake at ages 11, 13 and 15 years; age and sex differences. Aust J Nutr Dietet 51:111-120.

Magarey AM, Boulton TJC. 1995. The Adelaide Nutrition Study 3. Food sources of nutrients at ages 11, 13 and 15 years. Aust J Nutr Dietet 52:124-130.

Magarey AM, Boulton TJC. 1995. The Adelaide Nutrition Study 4. Meal habits and distribution of energy and nutrients through the day at ages 11, 13 and 15 years. Aust J Nutr Dietet 52:132-139.

Magarey AM, Boulton TJC. 1995. The effects of differences in dietary fat on growth, energy, and nutrient intake from infancy to age 8 years. Acat Paediatr 84:146-150.

Boulton TJC, Magarey AM, Cockington RA. 1995. Tracking of serum lipids and dietary energy, fat and calcium intake from 1-15 years. Acta Paediatr. 84:1050-1055.

Boulton TJC, Magarey AM, Cockington RA. 1995. Serum lipids and apolipoproteins for 1-15 years: changes with age and puberty and relationships with diet, parental cholesterol, and family history of ischaemic heart disease. Acta Paediatr 84:1113-1118.

Magarey AM, Boulton TJC. 1996. The Adelaide Nutrition Study 5. Differences in energy, nutrient and food intake at ages 11, 13 and 15 years according to fathers' occupation and parents' educational level.

Papers, presentations and activities in support of this thesis

Invited papers

Diet and Osteoporosis
Osteoporosis SA, Update osteoporosis, seminar for health professionals
Adelaide, August 1994

Diet and Osteoporosis
Osteoporosis SA, Public lecture
Adelaide, August 1994

Calcium requirements in Australian Children
Australian College of Paediatrics, Annual Scientific Meeting, Adelaide, May 1995.

Adolescents-calcium and iron status - cause for concern?
Joint conference Australian Council for Responsible Nutrition and Discipline of Nutrition and Dietetics, The University of Newcastle: Optimal Nutrition for the Family, Terrigal, NSW,June 1995

Calcium alternatives to milk; Calcium absorption
osteoporosis SA, osteoporosis seminar series fro health professionals, Adealide, September 1995.

Oral presentations

Longitudinal monitoring of forearm bone mineral density changes during adolescence and parent-child associations.
ANZ Bone and Mineral Society Annual Scientific meeting, Brisbane, September 1994

Bone growth in adolescence 1. Changes in bone mineral content and densuity with age and association with contemporary and early nutrient intake.
Aust Paed Res Soc, Adelaide, May 1995

Bone growth in adolescence 2. Genetic influences measures by parent-child associations in bone mineral content and bone mineral density.
Aust Paed Res Soc, Adelaide, May 1995.

Poster

Bone mineral changes through puberty.
XV International Congress of Nutrition, Adelaide, Sept 1993.

Activities

Member of the National Health and Medical Research Council's Expert Panel on Calcium for the working party on Consumer Education and Food Labelling.
1990-1992.

BIBLIOGRAPHY

Abrams SA, Stuff JE. 1994. Calcium metabolism in girls: current dietary intakes lead to low rates of calcium absorption and retention during puberty. Am J Clin Nutr 60:739-743.

Allen LH. 1982. Calcium availability: a review. Am J Clin Nutr 35:783-808.

Aloia JF, Vaswani A, Ma R, Flaster E. 1995. To what extent is bone mass determined by fat-free or fat mass? Am J Clin Nutr 61:1110-1114.

American Academy of Paediatrics. 1978. Calcium requirements in infancy and childhood. Pediatrics 62:826-834.

Andon MB, Lloyd T, matkovic V. 1994 Supplementation trials with calcium citrate malate: evidenmce in favor of increasing calcium RDA during childhood and adolescence. J Nutr 124:1412S-1417S.

Armamento-Villareal R, Villareal DT, Avioli LV, Civitelli R. 1992. Estrogen status and heredity are major determinants of premenopausal bone mass. J Clin Invest 90:2464-2471.

Baran D, Sorenson A, Grimes J, Lew R, Karellas A, Johnson B, Roche J. 1989. Dietary modification with dairy products for preventing vertebral bone loss in premenopausal women: a three-year prospective study. J Clin Endocrinol 70:264-270.

Basiotis PP, Welsh SO, Cronin FJ, Kelsay JL, Mertz W. 1987. Number of days of food intake records required to estimate individual and group nutrient intakes with defined confidence. J Nutr 117:1638-1641.

Bass S, Pearce C, Formica C, Inge K, Hendrick E, Harding A, Seeman E. 1995. The effects of exercise before puberty on growth and mineral accrual in elite gymnasts. J Paediatr and Child Health. 31:A4

Beaton GH. 1994 Approaches to analysis of dietary data: relationship between planned analysis and choice of methodology. Am J Clin Nutr 59 (suppl):253S-261S.

Bingham S. 1987. The dietary assessment of individuals; methods, accuracy, new techniques and recommendations. Nutr Abstr Rev 57:705-742.

Bingham SA, Gill C, Welch A, et al. 1994. Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recals, food-frequency questionnaires and estimated-diet records. Br J Nutr 72:619-643.

Bishop NJ, De Priester JA, Cole TJ, Lucas A. 1992. Reference values for radial bone width and mineral content using single photon absorptiometry in healthy children aged 4-10 years. Acta Paediatr 81:463-468.

Bonjour JP, Theintz G, Buchs B, Slosman D, Rizzoli R. 1991. Critical years and stages of puberty for spinal and femoral bone mass accumulation during adolescence. J Clin Endocrinol Metab 73:555-563.

Boulton TJC. 1981. Nutrition in childhood and its relationships to early somatic growth, body fat, blood pressure, and physical fitness. Acta Paediatr Scand 284(suppl):1-85.

Boulton TJC, Cockington RA, Craig IH, Magarey AM, Mazumdar J. 1995a. A profile of heart disease risk factors and their relation to parent's education, father's occupation and history of heart disease in 844 South Australian families: The Adelaide Children's WHO Collaborative Study. Aust J Paed Child Health 31:200-206.

Boulton TJC, Magarey AM, Cockington RA. 1995b. Tracking of serum lipids from 1-15 yeras in relation to continuity of dietary energy, fat and calcium intake. Acta Paediatr 64:1050-1055.

Bull NL. 1988. Studies of the dietary habits, food consumption and nutrient intakes of adolescents and young adults. Wld Rev Nutr Diet 57:24-74.

Carbon RJ. 1992. Exercise, amenorrhoea and the skeleton. Br Med Bull 48:546-560.

Carter DR, Bouxsein ML, Marcus R. 1992. New approaches for interpreting projected bone densitometry data. J Bone Miner Res 7:137-145.

Chan GM. 1991. Dietary calcium and bone mineral status of children and adolescents. Am J Dis Chil 145:631-634.

Chan GM, Hoffman K, McMurray M. 1995. Effects of dairy products on bone and body composition in pubertal girls. J Paediatr 126:551-556.

Christian JC, Slemenda CW, Williams CJ, Johnston CC. 1990. Bone density: evidence for gene interactions. J Bone Miner Res 5:207.

Christiansen C, Rodbro P, Jensen H. 1975a. Bone mineral content in the forearm measured by photon absorptiometry. Scand J Clin Lab Invest 35:323-326.

Christiansen C, Rodbro P, Thoger Nielson C. 1975b. Bone mineral content and estimated total body calcium in normal children and adolescents. Scand J Clin Lab Invest 35:507-510.

Cleghorn DB, Polley KJ, Bellon MJ, Chatterton J, Baghurst PA, Nordin BEC. 1991. Fracture rates as a function of forearm mineral density in normal postmenopausal women: retrospective and prospective data. Calcif Tissue Int 49:161-163.

Consensus development conference on osteoporosis, April 1-2, 1993. Am J Med 95(suppl 5A):1S-78S.

Cooper C, Campion G, Melton LJ. 1992. Hip fractures in the elderly: a worldwide projection. Osteoporosis Int 2:285-289.

CSIRONET. 1980. Diaryan Program. CSIRO Division of Human Nutrition, Adelaide.

Cumming RG. 1990. Calcium intake and bone mass. A quantitative review of the evidence. Calcif Tissue Int 47:194-201.

Cummings SR, Black DM, Nevitt MC, Browner WS, Cauley JA, Genant HK. 1990. Appendicular bone density and age predict hip fracture in women. JAMA 263:665-668.

Cundy T, Cornish J, Evans MC, Gamble G, Stapleton J, Reid IR. 1995. Sources of interracial variation in bone mineral density. J Bone Miner Res 10:369-73.

Davee AM, Roden CJ, Adler RA. 1990. Exercise patterns and trabecular bone density in college women. J Bone Miner Res 5:245-250.

Davie MW, Haddaway MJ. 1994. Bone mineral content and density in healthy subjects and in osteogenesis imperfecta. Arch Dis Child 70:331-334.

Davies KM, Recker RR, Stegman MR, Heaney RP, Kimmel DB, Leist J. 1989. Third decade bone gain in women. J Bone Miner Res 4:S327(abst 838).

DCSH. Department of Community Services and Health. 1989. National dietary survey of schoolchildren (aged 10-15 years):1985, No 2 Nutrient intakes. Canberra: Australian Government Publishing Service.

De Schepper J, Derde MP, Van den Broeck M, Piepsz A, Jonckheer MH. 1991. Normative data for lumbar spine bone mineral content in children: influence of age, height, weight and pubertal stage. J Nuclear Med 32:216-220.

Dequeker J, Nijs J, Verstaeten A, Geusens P, Gevers G. 1987. Genetic determinants of bone mineral content at the spine and the radius, a twin study. Bone 8:207-209.

Dhuper S, Warren MP, brocks-Gunn J, Fox R. 1990. Effects of hormonal status on bone density in adolescent girls. J Clin Endocrinol Metab 71:1083-1088.

Duke PM, Litt IF, Gross RT. 1980. Adolescents' self-assessment of sexual maturation. Paediatr 66:918-920.

Eaton SB, Nelson DA. 1991. Calcium in evolutionary perspective. Am J Clin Nutr 54:281S-287S.

Eisman J. 1991. OsteoPPPorosis - Prevention, Prevention and Prevention. Aust NZ J Med 21: 205-209.

Eisman JA. 1995a. Efficacy of treatment of osteoporotic fractures. Am J Med 98(suppl 2A):17S-23S.

Eisman JA. 1995b Vitamin D receptor gene alleles and osteoporosis: and affirmative view. J Bone Miner Res 10:1289-1293.

Eisman JA, Sambrook PN, Kelly PJ, Pocock NA. 1991. Exercise and its interaction with genetic influences in the determination of bone mineral density. Am J Med 91(suppl 5B):5S-9S.

Eisman JA, Kelly PJ, Morrison NA, Pocock NA, Yeoman R, Birmingham J, Sambrook PN. 1993. Peak bone mass and osteoporosis prevention. Ostoporosis Int Supl 1:S56-S60.

Faulkner RA, Bailey DA, Drinkwater DT, Wilkinson A, Houston CS, McKay HA. 1993a. Regional and total body bone mineral content, bone mineral density, and total body tissue composition in children 8-16 years of age. Calcif Tissue Int 53:7-12.

Fehily AM, Coles RJ, Evans WD, Elwood PC. 1992. Factors affecting bone density in young adults. Am J Clin Nutr 56:579-586.

Felson DT, Zhang Y, Hannan MT, Anderson JJ. 1993. Effects of weight and body mass index on bone mineral density in men and women: the Framingham study. J Bone Miner Res 8:567-573.

Finkelstein JS, Neer RM, Biller BMK, Crawford JD, Klibanski A. 1992. Osteopenia in men with a history of delayed puberty. N Engl J Med 326:600-604.

Flicker L, Hopper J, Young D. 1992. Bone mineral density and body composition in adolescent and elderly female twins. Proc ANZBMS Ann Scientific Meeting Adelaide:36.

Forwood MR, Burr DB. 1993. Physical activity and bone mass: exercises in futility? Bone Mineral 21:89-112.

Friedlander AL, Genant HK, Sadowsky S,Byl NN, Gluer CC. 1995. A two-year5 program of aerobics and weight training enhances bone mineral density of young women. J Bone Miner Res 10:574-585.

Frost HM. 1990. Skeletal structural adaptations to mechanical usage (SATMU): 3. The hyaline cartilage modelling problem. Anat Rec 226:423-32.

Garn S. 1970. The earlier gain and the later loss of cortical bone. In: Nutritional perspective. Springfield IL: Thomas CC.

Garnero P, Borel O, Sornay-Rendu E, Delmas PD. 1995. Vitamin D receptor polymorphisms do not predict bone turnover and bone mass in healthy premenopausal women. J Bone Miner Res 10:1283-1288.

Genant HK, Faulkner KG, Gluer CC. 1991. Measurement of bone mineral density: current status. Am J Med 91(suppl 5B):49S-53S.

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

Gilchrist NL, Turner JG, Smart ET, Hook E, Sadler W, March R. 1994. The effect of dairy supplementation in teenage girls on bone density 12 months interim analysis. Proc Satellite meeting of International Council on Calcium Regulating Hormones, Perth. 81:abs 113.

Gilsanz V, Gibbens DT, Carlson M, Boechat MI, Cann CE, Schulz EE. 1988a. Peak trabecular vertebral density: a comparison of adolescent and adult females. Calcif Tissue Int 43:260-262.

Gilsanz V, Gibbens DT, Roe TF, et al.1988b. Vertebral bone density in children: effect of puberty. Radiology 66:847-850.

Gilsanz V, Roe TF, Mora S, Costin G, Goodman WG. 1991. Changes in vertebral bone density in black girls and white girls during childhood and puberty. N Engl J Med 325:1597-1600.

Gilsanz V, Boechat MI, Roe TF, Loro ML, Sayre JW, Goodman WG. 1994. Gender differences in vertebral body sizes in children and adolescents. Radiology 190:673-677.

Glastre C, Brillon P, David L, Cochat P, Meunier PJ, Delmar PD. 1990. Measurement of bone mineral content of the lumbar spine by dual energy X-ray absorptiometry in normal children; correlations with growth parameters. J Clin Endocrinol Metab 70:1330-1333.

Gordon CL, Halton JM, Atkinson SA, Webber CE. 1991. The contributions of growth and puberty to bone mass. Growth Dev Aging 55:257-262.

Gordon CL, Webber CE. 1993. Body composition and bone mineral distribution during growth in females. Can Assoc Radiol J 44:112-116.

Goslings WRO, Cole TJ, Prentice A, Bishop NJ. 1995. Rate of radial bone mineral accretion in healthy children. Acta Paediatr 84:383-7.

Greulich WW, Pyle SI. 1959. Radiographic atlas of skeletal development of the hand. 2nd ed. Stanford University Press, Stanford, California.

Grimston SK, Morrison K, Harder JA, Hanley DA. 1992. Bone mineral density during puberty in western Canadian children. Bone Mineral 19:85-96.

Gullberg B, Duppe H, Nilsson B, et al. 1993. Incidence of hip fractures in Malmo. Sweden (1950-1991). Bone 14 (suppl 1): S23-29.

Gunnes M. 1994. Bone mineral density in the cortical and trabecular distal forearm in healthy childen and adolescents. Acta Paediatr 83:463-467.

Gunnes M, Lehmann EH. 1995. Dietary calcium, saturated fat, fiber and vitamin C as predictors of forearm cortical and trabecular bone mineral density in healthy children and adolescents. Acta Paediatr 84:388-92.

Gunnes M, Lehmann EH. 1996. Physical activity and dietary constituents as predictors of forearm cortical and trabecular bone gain in healthy children and adolescents: a prospective study. Acta Paediatr Scand 85:19-25.

Gutin B, Kasper MJ. 1992. Can vigorous exercise play a role in osteoporosis prevention? A review. Osteoporosis Int 2:55-69.

Halioua L, Anderson JJ. 1990. Age and anthropometric determinants of radial bone mass in premenopausal Caucasian women: a cross-sectional study. Osteoporosis Int 1:50-55.

Halioua L, Anderson JJB. 1989. Lifetime calcium intake and physical activity habits: independent and combined effects on the radial bone of healthy pre-menopausal Caucasian women. Am J Clin Nutr 49:534-541.

Hamill PVV, Drizid TA, Johnson CL, et al. 1979. Physical growth: National Center for Health Statistics percentiles. Am J Clin Nutr 32:607-629.

Hansman CF.1962. Appearance and fusion of ossification centers i the human skeleton. Am J Roentgenol 88:476-482.

Hansen MA. 1994. Assessment of age and risk factors on bone density and bone turnover in healthy premenopausal women. Osteoporosis Int 4:123-128.
Harris SS, Dawson-Hughes B. 1994. Caffeine and bone loss in healthy postmenopausal women. Am J Clin Nutr 60:573-578.

Heaney RP. 1993a. Bone mass, nutrition,and other lifestyle factors. Am J Med 95(suppl 5A):29S-33S.

Heaney RP. 1993b. Nutritional factors in osteoporosis. Annu Rev Nutr 13:287-316.

Heaney RP. 1993c. Protein intake and the calcium economy. J Am Diet Assoc 93:1259-1260.

Heaney RP, Recker RR. 1982. Effects of nitrogen,phosphorus and caffeine on calcium balance in women. J Lab Clin Med 99:46-55.

Heaney RP, Weaver CM. 1992. Effect of plant constituents on food calcium absorbability. J Bone Miner Res 7:S136(abst 174).

Heaney RP, Davies KM, Recker RR, Packard PT. 1990a. Long-term consistency of nutrient intakes in humans. J Nutr 120:869-875.

Heaney RP, Weaver CM, Fitzsimmons ML, Recker RR. 1990b. Calcium absorptive consistency. J Bone Miner Res 5:1139-1142.

Heaney RP, Recker RR, Hinders SM. 1988a. Variability of calcium absorption. Am J Clin Nutr 47:262-264.

Heaney RP, Weaver CM, Recker RR. 1988b. Calcium absorbability from spinach. Am J Clin Nutr 47:707-709.

Heinonen A, Oja P, Kannus P, Sievanen H, Manttari A, Vuori I. 1993. Bone mineral density of female athletes in different sports. Bone Mineral 23:1-14.

Henderson RC. 1991. Assessment of bone mineral content in children. J Paed Orthop 11:314-317.

Henderson NK, Price RI, Cole JH, Gutteridge DH, Bhagat CI. 1995. Bone density in young women is associated with body weight and muscle strength but not dietary intakes. J Bone Miner Res 10:384-93.

Hernandez-Avila M, Stampfer MJ, Ravnikar VA, et al. 1993 Caffeine and other predictors of bone density among pre- and perimenopausal women. Epidemiology 4:128-134.

Heuter C. 1862. Anatomische studien an der extremitatengelenken neugeborner und erwachsener. Virchows Arch 25:572.

Hirota T, Nara M, Ohguri M, Manago E, Hirota K. 1992. Effect of diet and lifestyle on bone mass in Asian young women. Am J Clin Nutr 55:1168-1173.

Horsman A, Leach AE. 1974. The estimation of the cross-sectional area of the ulna and radius. Am J Phys Anthropol 40:173-185.

Hui SL, Johnston CC, Mazess RB. 1985. Bone mass in normal children and young adults. Growth 49:34-43.

Hui SL, Slemenda CW, Johnston CC. 1990. The contribution of bone loss to postmenopausal osteoporosis. Osteoporosis 1:30-34.

Hustmeyer FG, Peacock M, Hui S, Johnston CC, Christian JC. 1994. Bone mineral density in relation to polymorphisms at the vitamin D receptor locus. J Clin Invest 94:2130-2134.

Jaworski C. 1981. Physiology and pathology of bone remodelling. Orth Clin Nth Amer 12:485-512.

Jelic T, Wardlaw GM, Ilich JZ et al. 1992. Timing of peak bone mass in Caucasian females. J Bone Min Res 7S:S139(abs 187).

Johnell O, Nilsson B, Obrant K, Sembo I. 1984. Age and sex patterns of hip fracture - change in 30 years. Acta Orthop Scand 55:290-292.

Johnell O, Gullberg B, Allander E, Kanis JA. 1992. The apparent incidence of hip fractures in Europe: a study of national register sources. Osteoporos Int 2:298-302.

Johnston CC, Miller JZ, Slemenda CW, Reister TK, Hui S, Christian JC, Peacock M. 1992. Calcium supplementation and increases in bone mineral density in children. N Engl J Med 327:82-87.

Kanders B, Dempster DW, Lindsay R. 1988. Interaction of calcium nutrition and physical activity on bone mass in young women. J Bone Miner Res 3:145-149.

Kanis JA, Passmore R. 1989. Calcium supplementation of the diet - I. Br Med J 298:137-140.

Karlsson MK, Gardsell P, Johnell O, Nilsson BE, Akesson K, Obrant KJ. 1993. Bone mineral normative data in Malmo, Sweden. Comparison with reference data and hip fracture incidence in other ethnic groups. Acta Orthop Scand 64:168-172.

Katzman DK, Bachrach LK, Carter DR, Marcus R. 1991. Clinical and anthropometric correlates of bone mineral acquisition in healthy adolescent girls. J Clin Endocrinol Metab 73:1332-1339.

Kelly PJ, Hopper JL, Macaskill GT, Pocock NA, Sambrook PN, Eisman JA. 1990a. Genetic factors in bone turnover. J Clin Endocrinol Metab 72:808-814.

Kelly PJ, Pocock NA, Sambrook PN, Eisman JA. 1990b. Dietary calcium, sex hormones and bone mineral density in normal men. Br Med J 300:1361-1364.

Kelly PJ, Twomey L, Sambrook PN, Eisman JA. 1990c. Sex differences in peak adult bone mineral density. J Bone Miner Res 5:1169-1175.

Kelly PJ, Nguyen T, Hopper J, Pocock N, Sambrook P, Eisman J. 1993. Changes in axial bone density with age: a twin study. J Bone Miner Res 8:11-17.

Kirk S, Sharp CF, Elbaum N, Endres DB. 1989. Effect of long-distance running on bone mass in women. J Bone Miner Res 4:515-522.

Kleerekoper M, Tolia K, Parfitt AM. 1981. Nutritional, endocrine, and demographic aspects of osteoporosis. Orthop Clin Nth America 12:547-558.
Krabbe S, Christiansen C. 1984. Longitudinal study of calcium metabolism in male puberty I Bone mineral content, and serum levels of alkaline phosphatase, phosphate and calcium. Acta Paediatr Scand 73:745-750.

Krabbe S, Christiansen C, Rodbro P, Transbol I. 1979. Effect of puberty on rates of bone growth and mineralisation. Arch Dis Chil 54:950-953.

Krabbe S, Christiansen C, Rodbro P, Transbol I. 1980. Pubertal growth as reflected by simultaneous changes in bone mineral content and serum alkaline phosphatase. Acta Paediatr Scand 69:49-52.

Krabbe S, Hummer L, Christiansen C. 1984. Longitudinal study of calcium metabolism in male puberty II Relationship between mineralization and serum testosterone. Acta Paediatr Scand 73:750-755.

Krall EA, Dawson-Hughes B. 1993. Heritable and life-style determinants of bone mineral density. J Bone Miner Res 8:1-9.

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 Mineral 17:75-85.

Kroger H, Kotaniemi A, Kroger L, Alhava E. 1993. Development of bone mass and bone density of the spine and femoral neck - a prospective study of 65 children and adolescents. Bone Mineral 23:171-182.

Landin L, Nilsson BE. 1981. Forearm bone mineral content in children - Normative data. Acta Paediatr Scand 70:919-923.

Lau EMC. 1993. Hip fracture in Asia - trends, risk factors and prevention In: Christiansen C, Riis B, eds Osteoporosis proceedings fourth international symposium on osteoporosis, Rodovse, p58-61.

Lee WT, Leung SS, Ng MY, Wang SF, Xu YC, Zeng WP, Lau J. 1993. Bone mineral content of two populations of Chinese children with different calcium intakes. Bone Mineral 23:195-206.

Lee WT, Leung SS, Wang SH, et al 1994. Double-blind, controlled calcium supplementation and bone mineral acretion in children accustomed to a low-calcium diet. Am J Clin Nutr 60:744-750.

Lewis AF. 1981. Fracture of the neck of femur: changing incidence. BMJ 283: 1217-1220.

Li K, Zernicke RF, Barnard J, Li AF. 1991. Differentail response of rat limb bones to strenuous exercise. J Appl Physiol 70:554-560.

Livingstone MBE, Prentice AM, Coward WA. 1992. Validation of estimates of energy intake by weighed dietary record and diet history in children and adolescents. Am J Clin Nutr 56:29-35.

Lloyd T, Andon MB, Rollings N, et al.1993 . Calcium supplementation and bone mineral density in adolescent girls. JAMA 270:841-844.

Lloyd T, Rollings N, Andon MB, et al. 1992. Determinants of bone density in young women.1.Relationships among pubertal development, total body bone mass, and total bone density in premenarchal females. J Clin Endocrinol Metab 75:383-387.

Lu PW, Briody JN, Ogle GD et al. 1994. Bone mioneral density of total body, spine, and femoral neck in children and young adults: a cross-sectional and longitudinal study. J Bone Miner Res 9:1451-1458.

Lu PW, Cowell CT, Lloyd-Jones SA. 1995. Volumetric bone density of femoral shaft in normal subjects aged 8 to 27 years. Bone 16 (suppl): abstract 245.

Lutz J. 1986. Bone mineral, serum calcium and dietary intakes of mother/daughter pairs. Am J Clin Nutr 44:99-106.

Magarey AM, Boulton TJC. 1987. Percentiles of food energy, macro, and micronutrient intake from infancy to age 8 years. Med J Aust 147:124-127.

Magarey AM, Boulton TJC. 1994a. The Adelaide Nutrition Study 1. Food energy intake through adolescence: including an evaluation of the problem of under-recording, age and sex differences. Aust J Nutr Diet 51:104-110.

Magarey AM, Boulton TJC. 1994b. The Adelaide Nutrition Study 2. Macronutrient and micronutrient intakes at age 11, 13 and 15 years, age and sex differences. Aust J Nutr Diet 51:111-120.

Magarey AM, Boulton TJC. 1995a. The Adelaide Nutrition Study 3. Food sources of nutrients at ages 11, 13 and 15 years. Aust J Nutr Dietet 52:104-110.

Magarey AM, Boulton TJC. 1995a. The Adelaide Nutrition Study 4. Meal habits and distribution of energy and nutrients through the day at ages 11, 13 and 15 years. Aust J Nutr Diet 52:111-120.

Margen S, Chu J-Y, Kaufman NA, Callaway DH. 1974. Studies in calcium metabolism. I. The calciuretic effect of dietary protein. Am J Clin Nutr 27:584-589.

Massey LK, Whiting SJ. 1993. Caffeine, urinary calcium, calcium metabolism and bone. J Nutr 123:1611-1614.

Matkovic V. 1991. Calcium metabolism and calcium requirements during skeletal modelling and consolidation of bone mass. Am J Clin Nutr 54:245S-260S.

Matkovic V, Heaney RP. 1992. Calcium balance during human growth: evidence for threshold behavior. Am J Clin Nutr 55:992-996.

Matkovic V, Ilich JZ. 1993. Calcium requirements for growth: are current recommendations adequate? Nutr Rev 51:171-180.

Matkovic V, Kostial K, Simonovic I, Buzina R, Brodarec A, Nordin BEC. 1979. Bone status and fracture rates in two regions of Yugoslavia. Am J Clin Nutr 32:540-549.

Matkovic V, Fontana D, Tominac C, Goel P, Chestnut CH. 1990. Factors that influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. Am J Clin Nutr 52:878-888.

Matkovic V, Jelic T, Wardlaw GM et al . 1994. Timing of peak bone mass in Caucasian females and its implications for the prevention of osteoporosis. J Clin Invest 93:799-808.

Mazess RB. 1982. On aging bone loss. Clin Orthop Rel Res 165:239-252.

Mazess RB, Barden HS. 1990. Interrelationships among bone densitometry sites in normal young women. Bone Mineral 11:347-56.

Mazess RB, Barden HS. 1991. Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking, and birth-control pills. Am J Clin Nutr 53:132-142.

Mazess RB, Cameron JR.1971. Skeletal growth in school children: maturation and bone mass. Am J Physic Anthropol 35:399-407.

Mazess RB, Barden HS, Drinka PJ, Bauwens SF, Orwoll ES, Bell NH. 1990. Influence of age and body weight on spine and femur bone mineral density in white men. J Bone Miner Res 5:645-652.

Mc Culloch RG, Bailey DA, Houston CS, Dodd BL. 1990. Effects of physical activity, dietary calcium intake and selected lifestyle factors on bone density in young women. Can Med Assoc J 142:221-227.

McCormick DP, Ponder SW, Fawcett HD, Palmer JL. 1991. Spinal bone mineral density in 335 normal and obese children and adolescents: evidence for ethnic and sex differences. J Bone Miner Res 6:507-513.

McKay HA, Bailey DA, Wilkinson AA, Houston CS. 1994. Familial comparison of bone mineral density at the proximal femur and lumbar spine. Bone Mineral 24:95-107.

Melhus H, Kindmark A, Amer S, Wilen B, Lindh E, Ljunghall S. 1994. Vitamin D receptor genotypes in osteoporosis. Lancet 344:949.

Melton LJ. 1993. Hip fractures: a worldwide problem today and tomorrow. Bone 14:S1-S8.

Metz JA, Anderson JJB, Gallagher PN. 1993. Intakes of calcium, phosphorus, and protein, and physical activity level are related to radial bone mass in young adult women. Am J Clin Nutr 58:537-542.

Miller JZ, Johnston CC. 1990. Relationship of dietary calcium and bone mass in twin children. J Bone Miner Res 5(suppl):S275(abs 805).

Miller JZ, Slemenda CW, Melton LJ, Reister TK, Hui S, Johnston CC. 1991. The relationship of bone mineral density and anthropometric variables in healthy male and female children. Bone Mineral 14:137-152.

Mizuta T, Benson WM, Foster BK, paterson DC, Morris LL. 1987. Statistical analysis of the incidence of physeal injuries. J Pediatr Orthop 7:518-523.
Mora S, Goodman WG, Loro ML, Roe TF, Sayre J, Gilsanz V. 1994. Age-related changes in cortical and cancellous vertebral bone density in girls: assessment with quantitative CT. Am J Roentgenol 162:405-409.

Moreira-Andres MN, del Canizo FJ, Garcia L, Godoy G, Puente MD, Hawkins F. 1994. Serum insulin-like growth factor-I and bone mineral density correlations in healthy children. Proceedings Int Symp Growth Hormone and Growth Factors in Endocrinology and Metabolism:Gothenburg. p99.

Morrison NA, Yeoman R, Kelly PJ, Eisman JA. 1992. Contribution of trans-acting factor alleles to normal physiological variability: Vitamn D receptor gene polymorphisms and circulating osteocalcin. Proc Natl Acad Sci USA 89:6665-6669.

Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. 1994. Prediction of bone density from vitamin D receptor alleles. Nature 367:284-287.

Mosekilde L. 1993. Normal age-related changes in bone mass, structure, and strength - consequences of the remodelling process. Dan Med Bull 1:65-83.

Need AG, Horowitz M, Walker CJ, Chatterton BE, Chapman IC, Nordin BEC. 1989. Cross-over study of fat corrected forearm mineral content during nandrolene decanoate therapy for osteoporosis. Bone 10:3-6.

Nelson M, Black AE, Morris JA, Cole TJ. 1989 Between- and within-subject variation in nutrient intake from infancy to old age: estimating the number of days required to rank dietary intakes with desired precision. Am J Clin Nutr 50:155-67.

Nelson D, Feingold M, Mascha E, Kleerekoper M. 1992. Comparison of single-photon and dual-energy x-ray absorptiometry of the radius. Bone Mineral 18:77-83.

Nguyen T, Sambrook P, Kelly P, Jones G, Lord S, Freund J, Eisman J. 1993. Prediction of osteoporotic fractures by postural instability and bone density. Br Med J 307:1111-1115.

NHMRC. National Health and Medical Research Council. 1989. Recommended dietary intakes for use in Australia. Australian Government Publishing Service, Canberra.

Nieves JW, Cosman F, Mars C, Lindsay R. 1992. Comparative assessment of bone mineral density of the forearm using single photon and dual X-ray absorptiometry. Calcif Tissue Int 51:352-355.

NIH 1994. NIH Consensus Development Panel on Optimal Calciumk Intake. JAMA 272:1942-1948.

Nilas L, Podenphant J, Riis BJ, Gotfiedsen A, Christiansen C. 1987 Usefulness of regional bone measurements in patients with osteoporotic fractures of the spine and distal forearm. J Nucl Med 28:960-65.

Nishiyama S, Inomoto T, Tomoeda S, Nakamura T, Matsuda I. 1994. Study of spinal bone mineral density in normal Japanese children. Proceedings Int Symp Growth Hormone and Growth Factors in Endocrinology and Metabolism:Gothenburg. p98.

Nordin BEC. 1993. Bone mass, bone loss, bone density and fractures. Osteoporosis Int Suppl 1:S1-7.

Nordin BEC. 1990. Calcium. In Recommended Nutrient Intakes Australian Papers. Ed Truswell AS. Australian Professional Publishers, Sydney.

Nordin BEC, Polley KJ. 1987. Metabolic consequences of the menopause. Calcif Tissue Int 41 (suppl 1):s1-s59.

Nordin BEC, Chatterton BE, Steurer TA, Walker CJ. 1986. Forearm bone mineral content does not decline with age in premenopausal women. Clin Orthop Rel Res 211:252-256.

Nordin BEC, Cleghorn DB, Chatterton BE, Morris HA, Need AG. 1993. A 5-year longitudinal study of forearm bone mass in 307 postmenopausal women. J Bone Min Res 8:1427-1432.

Norusis MJ. 1988. SPSS/PC+ V2.0. SPSS Inc Chicago.

Nowson CA, Sherwin AJ, Green RM. 1992. Bone density, calcium intake and activity in female twins. Proc Nutr Soc Aust 17:232.

Nowson CA, Green RM, Guest CS. 1994. The effect of calcium supplementation for one year on bone mass in adolescent female twins. Proc ANZBMS Annual Scientific Meeting Brisbane 24.

NRC. National Research Council. 1989. Recommended Dietary Allowances,10th ed. National Academy Press, Washington: DC.

Ogle GD, Allen JR, Humphries IRJ et al. 1995. Body composition assessment by dual x-ray absorptiometry in subjects aged 4-26y. Am J Clin Nutr 61:746-753.

Ogden JA. 1982. Skeletal growth mechanism injury patterns. J Pediatr Orthop 2:371-377.

Pan H, Radcliffe SG. 1992. A new method of deriving velocity and acceleration curves for height from the kernel estimation of distance. Ann Human Biol 19:303-316.

Parfitt AM. 1987. Trabecular bone architecture in the pathogenesis and prevention of fracture. Am J Med 82(suppl1B):68-72.

Patel DN, Pettifor JM, Becker PJ, Grieve C, Lescher K. 1992. The effect of ethnic group on appendicular bone mass in children. J Bone Miner Res 7:263-272.

Paul AA, Southgate DAT. 1985. The Composition of Foods. Her Majesty's Stationery Office, London.

Peacock M. 1991. Calcium absorption efficiency and calcium requirements in children and adolescents. Am J Clin Nutr 54:261S-265S.

Peacock M. 1995. Vitamin D receptor gene alleles and osteoporosis: a contrasting view. J Bone Miner Res 10:1294-1297.

Penfold JL, Boulton TJC, Thomsett MJ, et al 1980. Hormonal profile of puberty in South Australian children II. Aust Paediatr J 16:17-23.

Petrie RS, Sinaki M, Squires RW, Bergstralh EJ. 1993. Physical activity, but not aerobic capacity correlates with back strength in healthy premenopausal women from 29 to 40 years of age. Mayo Clin Proc 68:738-742.

Pocock NA, Eisman JA, Hopper JL. 1987. Genetic determinants of bone mass in adults. A twin study. J Clin Invest 80:706-710.

Pollitzer WS, Anderson JJB. 1989. Ethnic and genetic differences in bone mass: a review with a hereditary vs environmental perspective. Am J Clin Nutr 50:1244-1259.

Ponder SW, McCormick DP, Fawcett HD, Palmer SL, McKernan MG, Brouhard BH. 1990. Spinal bone mineral density in children aged 5.00 through 11.99 years. Am J Dis Child 144:1346-1348.

Preece MA, Pan H, Radcliffe SG. 1992. Auxological aspects of male and female puberty. Acta Paediatr Suppl 383:11-13.

Recker MD, Davies M, Hinders SM, Heaney R, Stegman MR, Kimmel DB. 1992. Bone gain in young adult women. JAMA 268:2403-3408.

Reid IR, Ames RW, Evans MC, Gamble GD, Sharpe SJ. 1993. Effect of calcium supplementation on bone loss in postmenopausal women. N Engl J Med 328:460-464.

Reid IR, Chin K, Evans MC, Jones JG. 1994. Relation between increases in length of hip axis in older women between 1950s and 1990s and increase in age rates of hip fracture. Br Med J 309:508-509.

Rico H, Revilla M, Hernandez ER, Villa LF, Alvarez del buergo M. 1992. Sex differences in the acquisition of total bone mineral mass peak assessed through dual-energy x-ray absorptiometry. Calcif Tissue Int 51:251-254.

Rico H, Revilla M, Hernandez ER, Gomez-Castresana F, Villa LF. 1993a. Bone mineral content and body composition in postpubertal cyclist boys. Bone 14:93-95.

Rico H, Revilla M, Villa LF, Alvarez-de-Buergo M. 1993b. Age-related differences in total and regional bone mass: a cross-sectional study with DXA in 429 normal women. Osteoporosis Int 3:154-159.

Rico H, Revilla M, Villa LF, Hernandez ER, Alvarez de Buergo M, Villa M. 1993c. Body composition in children and Tanner's stages: a study with dual-energy x-ray absorptiometry. Metabolism 42:967-970.

Rico H, Revilla M, Villa LF, Alvarez-de-Buergo M, Ruiz-Contreras D. 1994. Determinants of total-body and regional bone mineral content and density in postpubertal normal women. Metabolism 43:263-266.

Riis BJ, Krabbe S, Christiansen C, Catherwood BD, Deftos LJ. 1985. Bone turnover in male puberty: a longitudinal study. Calcif Tissue Int 37:213-217.

Rockwood PR, Horne JG, Cryer C. 1990. Hip fractures: a future epidemic? J Orthop Trauma 4:388-393.

Rothman KJ. 1986. Modern epidemiology. Little Brown and Company, Boston/Toronto.

Rubin K, Schidvan V, Gendrau P, Sarfrazi M, Mendola R, Dalsky G. 1993. Predictors of axial and peripheral bone mineral density in healthy children and adolescents, with special attention to the role of puberty. J Pediatr 123:863-870.

Russell-Aulet M, Wang J, Thornton J, Colt E, Pierson R. 1991. Bone mineral density and mass by total body dual-photon absorptiometry in normal white and Asian men. J Bone Miner Res 6:1109-1113.

Rutherford OM. 1993. Spine and total body bone mineral density in amenorrheic endurance athletes. J Appl Physiol 74:2904-2908.

Salkeld G, Leeder S. 1990. Osteoporosis and its cost to Australia. Public Health Assoc. conference, Hobart, Tasmania.

Schlenker RA, Von Seggen WW. 1976. The distribution of cortical and trabecular bone mass along the lengths of the radius and ulna and the implications for in vivo bone mass measurements. Calcif Tissue Res 20:41-52.

Seeman E. 1993. Osteoporosis in men: epidemiology, pathophysiology and treatment possibilities. Am J Med 95(suppl5A):22S-28S.

Seeman E, Hopper JL, Bach LA, Cooper ME, Parkinson E, McKay J, Jerums G. 1989. Reduced bone mass in daughters of women with osteoporosis. N Engl J Med 320:554-558.

Sentipal JM, Wardlaw GM, Mahan J, Matkovic V. 1991. Influence of calcium intake and growth indices on vertebral bone mineral density in young females. Am J Clin Nutr 54:425-428.

Shaw NJ, Bishop NJ. 1995. Mineral accretion in growing bones - a framework for the future? Arch Dis Chil 72:177-179.

Slemenda CW, Johnston CC. 1993. High intensity activities in young women: site specific bone mass effects among female figure skaters. Bone Mineral 20:125-132.

Slemenda CW, Hui SL, Longcope C, Johnston CC. 1989. Cigarette smoking, obesity, and bone mass. J Bone Miner Res 4:737-741.

Slemenda CW, Hui SL, Johnston CC, Christain JC, Williams CJ, Meaney FJ. 1990. Bone mass and anthropometric measurements in adult females. Bone Mineral 11:101-109.

Slemenda CW, Christian JC, Williams CJ, Norton JA, Johnston CCJr. 1991a. Genetic determinants of bone mass in adult women: a reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. J Bone Miner Res 6: 561-567.

Slemenda CW, Miller JZ, Hui SL, Reister TK, Johnston CCJr. 1991b. Role of physical activity in the development of skeletal mass in children. J Bone Miner Res 6:1227-1233.

Slemenda CW, Reister TK, Peacock M, Johnston CC. 1993. Bone growth in children following the cessation of calcium supplementation. J Bone Miner Res 8:S154(abs 151).

Smith DM, Nance WE, Kang KW, Christian JC, Johnsont CC. 1973. genetic factors in determining bone mass. J Clin Invest 52:2800-2808.

Smith EL, Gilligan C, Smith PE, Sempos CT. 1989. Calcium supplementation and bone loss in moddle-aged women. Am J Clin Nutr 50:833-842.

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. J Bone Miner Res 5:589-595.

Snow-Harter C, Bouxsein ML, Lewis BT, Carter DR, Marcus R. 1992a. Effects of resistance and endurance exercise on bone mineral status of young women: A randomized exercise intervention trial. J Bone Miner Res 7:761-769.

Snow-Harter C, Whalen R, Myburgh K, Arnaud S, Marcus R. 1992b. Bone mineral density, muscle strength, and recreational exercise in men. J Bone Miner Res 7:1291-1296.

Southard RN, Morris JD, Mahan JD, Hayes JR, Torch MA, Sommer A, Zipf WB. 1991. Bone mass in healthy children: measurement with quantitative DXA. Radiology 179:735-738.

Sowers MF, Wallace RB, Lemke JR. 1985. Correlates of forearm bone mass among women during maximal bone mineralisation. Prev Med 14:585-596.

Sowers MF, Kshirsagar A, Crutchfield M, Updike S. 1991. Body composition, age and femoral bone mass of young adult women. Ann Epidemiol 1:245-254.

Spector TD, Cooper C, Lewis AF. 1990. Trends in admission for hip fracture in England and Wales 1968-85. Brit Med J 300:1173-1174

Stegman M, Heaney R, Recker R. 1990. Early bone mineral measurement. A predictor of later appendicular fracture. J Bone Miner Res 5(suppl):S117(abst 174).

Steinhaus A. 1933. Chronic effects of exercise. Physiol Rev 13:103-47. cited in Forwood and Burr 1993.

Takeshita T, Yamagata Z, Tijima S, Nakamura T, Ouchi Y, Orima H, Asaka A. 1992. Genetic and environmental factors of bone mineral density indicated in Japanese twins. Gerentology 38 (suppl 1):43-49.

Tanner JM.1962. Growth at adolescence, ed 2. Oxford, Blackwell Scientific Publications.

Tanner JM, Davies PSW. 1985. Clinical longitudinal standards for height and weight velocities for North American children. J Paediatr 107:317-329.

Teegarden D, Proulx WR, Martin BR et al 1995. peak bone mass in tyoung women. J Bone Miner Res 10:711-715.

Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H, Sizonenko PC, Bonjour JP. 1992. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab 75:1060-1065.

Thomas KA, Cook SD, Bennett JT, Whitehead TS, Rice JC. 1991. Femoral neck and lumbar spine bone mineral densities in a normal population 3-20 years of age. J Paediatr Orthop 11:48-58.

Tokita A, Kelly P, Nguyen T. 1992. Genetic determinants of type II collagen degradation and synthesis: further evidence for genetic regulation of bone turnover. Proc ANZBMS Ann Scientific Meeting Adelaide:35.

Trotter M, Hixon BB. 1974. Sequential changes in weight, density, and percentage ash weight of human skeletons from an early fetal period through old age. Anat Rec 179:1-18.

Trouerbach WT, de Man SA, Gommens D, Zwamborn AW, Grobbee DE. 1991. Determinants of bone mineral content in childhood. Bone Mineral 13:55-67.

Tsukahara H, Sudo M, Umezaki M, Hiraoka M, Yamamoto K, Isshi Y, Haruki S. 1992. Dual-energy X-ray absorptiometry in the lumbar spine, proximal femur and distal radius in children. Pediatr Radiol 22:560-562.

Turner JG, Gilchrist NL, Ayling EM, Hassall AJ, Hooke EA, Sadler WA. 1992. Factors affecting bone mineral density in high school girls. NZ Med J 105:95-96.

Tylavsky FA, Bortz AD, Hancock RL, Anderson JJB. 1989. Familial resemblance of radial bone mass between premenopausal mothers and their college-age daughters. Calcif Tissue Int 45:265-272.

Tylavsky FA, Anderson JJB, Talmage RV, Taft TN. 1992. Are calcium intakes and physical activity patterns during adolescence related to radial bone mass of white college-age females? Osteoporos Int 2:232-240.

Valimaki MJ, Karkkainen M, Lamberg-Allardt C. 1994. Exercise, smoking, and calcium intake during adolescence and early adulthood as determinants of peak bone mass. Brit Med J 309:230-235.

Volkman R. 1862. Chirurgische ertahrungen uber knochenverbiegungen und knochenwachsthum. Arch Pathol Anat 24:512.

Wallace WA. 1983. The increasing incidence of fracture of the proximal femur: an orthopaedic epidemic. Lancet i:1413-1414.

Weaver CM, Martin BR, Plawecki KL, et al 1995. Differences in calcium metabolism between adolescent and adult females. Am J Clin Nutr 61:577-81.

Welkowitz J, Ewen RB, Cohen J. 1982. Introductory statistics for the behavioural sciences. Orlando FL:Aademic Press.

Whalen RT, Carter DR, Steele CR. 1988. Influence of physical activity on the regulation of bone density. J Biomech 21:825-37.

Williams JA, Wegner J, Wasnich R, Heilbrun L. 1984. The effect of long-distance running upon appendicular bone mineral content. Med Sci Sports exercise 16:223-227.

Wishart JM, Horowitz M, Cochran M, Need AG, Chatterton BE, Nordin BEC. 1991. Cross-sectional and longitudinal study of bone mineral content of the distal forearm in adult premenopausal women. Horm Metab Res 23:185-7.

Yamagata Z, Miyamura T, Tijima S, Asaka A, Sasaki M, Kato J, Koizumi K. 1994. Vitamin D receptor gene polymorphism and bone mineral density in healthy Japanese women. Lancet 344:1027.

Zetterberg C, Anderson GBJ. 1980. Fractures of the proximal radius and of the femur in Gotheneberg, Sweden 1940-1979. Acta Orthopaed Scand 54:681-686.