Nutrition Research Reviews (2001), **14**, 119–151 DOI: 10.1079/NRR200120

Improvement of bone health in childhood and adolescence

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Osteoporosis as a worldwide problem is discussed in the present review and the question of improving peak bone mass to reduce the risk of osteoporosis and osteoporotic fracture is addressed. The available evidence points to pre-puberty and puberty as the most opportune periods for intervention, but the potential for achievable increments in bone mass is shown to be small compared with the overwhelming influence of heredity, body composition and hormonal factors on bone. Lean body mass appears to be positively correlated with bone mass, while black–white racial differences in bone mass appear to be related to greater lean mass and lower bone turnover rate in blacks. Within races, twin and parent–offspring models have suggested that 46–80 % of the variance in bone mineral density can be explained by inherited factors; however, the mechanism of the genetic influence on bone density remains poorly understood. Moderate regular exercise seems to maintain bone mass while more vigorous regular exercise increases it in children and young adults. Ca intake has been found to be positively associated with bone mass in many but not all studies, possibly because of a ceiling at about 1300–1500 mg/d for young people. Other nutritional variables, including vitamin D, have been little investigated in relation to childhood and adolescent bone mass. The influence of milk as a source of highly bioavailable Ca and other nutrients has also been less frequently investigated, which is of concern given the cessation of school milk programmes in Western countries over the last three decades. Intervention studies to improve bone health in young people have mainly been based on Ca milk or exercise. The evidence points to the benefits to bone of such interventions, particularly when commenced pre-puberty, and it seems that daily consumption of 200–300 ml milk/d by children and adolescents has no adverse side effects. The benefits to bone are almost universally shown to be lost fairly rapidly after Ca or exercise intervention

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Abbreviations: BMC, bone mineral content; BMD, bone mineral density; DPA, dual-photon absorptiometry; DXA, dual-energy X-ray absorptiometry; SPA, single-photon absorptiometry; QCT, quantitative computed tomography; VDR, vitamin D receptor; $1,25(OH)_2D$, 1α,25-dihyroxycholecalciferol; 25(OH)D, 25-hydroxycholecalciferol.

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ceases; there is therefore no justification in terms of bone health for short-term interventions of this nature. The question of withdrawal of milk supplementation has undergone very little examination. Further, very little evidence is available on the effects of long-term interventions of any sort on bone health. Nevertheless, the data obtained so far permit the suggestion that promotion of Ca intake (e.g. at the higher level of current recommendations) and exercise commencing in the pre-pubertal period should be adopted as policy now.

Bone: Calcium: Osteoporosis: Adolescence

Introduction

Bone mass at any time during adult life is determined by peak bone mass achieved at maturity and bone loss with ageing. Low bone mass is considered to increase the risk of clinical osteoporosis, which is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent susceptibility to fracture, especially in the wrist, lumbar spine and hip regions (Lau & Cooper, 1996). Osteoporosis is a serious public health problem. About 75 % of all fractures in women older than 45 years in developed countries are related to osteoporosis and these fractures are a significant cause of morbidity and mortality (Cummings, 1985). Since the most susceptible population, the elderly, is expanding worldwide, the frequency of osteoporosis and osteoporosis-related fractures is expected to increase. For example, while the rates of osteoporosis in Asia are low by world standards, they are rising rapidly and total cases in Asia are likely to outstrip those in Western countries by 2050 due to the rapid ageing of Asian societies. Despite the very low incidence rate of hip fracture in Beijing in 1988, it had increased 34 % in women and 33 % in men by 1992 (Xu *et al*. 1996). Lau & Cooper (1996) have documented the increases in hip fracture in several Asian populations in the last three decades. While only 30 % of the world's hip fractures occurred in Asia in 1990, Cooper *et al*. (1992) estimated that over 50 % of hip fractures would occur in Asia by 2050.

There is a growing emphasis on osteoporosis prevention, because actual therapy for this disease is difficult. A maximal bone mass at skeletal maturity is considered to be the best protection against age-related bone loss and subsequent fracture risk (Matkovic *et al*. 1990). Small increases in peak bone mass could lead to decreases in the rate of fractures later in life. According to prospective studies of the relationship between bone mass and the incidence of subsequent fractures in middle-aged and elderly women, a change of 1 SD in bone mass may alter the risk of fracture by as much as 100 % (Hui *et al*. 1989; Wasnich *et al*. 1989). Therefore, the study of peak bone mass attainment in adolescence is of great importance for identifying risk factors in early life and for developing prophylactic protocols to prevent osteoporosis. We are particularly interested in peak bone mass achievement in children accustomed to low Ca intakes, such as those consuming predominantly plant-based diets in Asia, because of the concerns expressed earlier about the impending osteoporosis catastrophe in this part of the world.

In the present review, the relationship between growth and bone mass and factors affecting bone mass accumulation, and the suitability of interventions in adolescence, including milk supplementation, will be discussed. The discussion will be divided into two broad sections, one assessing the literature on measured factors responsible for determining bone mass in adolescence, and the second assessing deliberate interventions to improve bone mass in this period.

Bone physiology and bone mass measurement

Bone structure and function, bone growth, and bone mass measurement are summarised here first, because they are essential for understanding bone mass accumulation in adolescence.

Bone physiology

Bone is composed of an organic protein-based matrix in which bone minerals are deposited. Two-thirds of the weight of bone is due to minerals and the remainder is collagen and water. Most (95 %) of the organic matrix of bone is constituted of collagen I. The remaining 5 % is composed of minor quantities of other types of collagen (III, V, VI, and VII) and several non-collagenous components. The solid mineral phase of bone consists of small hexagonal crystals, which are plate-like in shape and of variable size. The main constituents of the crystalline salts are Ca and phosphate in the form of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). The skeleton consists of two morphologically different forms of bone: cortical (compact) and cancellous or trabecular (spongy) bone. Cortical bone is dominant in the bones of the extremities, like the femur and tibia, whereas cancellous bone is dominant in the axial skeleton, like the vertebrae and pelvis. Cortical bone makes up 80 % of the skeleton and cancellous bone 20 %. Cortical bone provides rigidity and is mainly responsible for mechanical and protective functions. Cancellous bone provides elasticity. It is metabolically more active, and responsible for approximately 50 % of skeletal metabolism. The main function of bone is skeletal support and protection of internal organs. It also plays an important role in maintaining Ca homoeostasis in the body through bone formation and resorption (Marks & Hermey, 1996).

Bone metabolism is characterized by two metabolic processes: formation of new bone by osteoblasts and degradation (resorption) of old bone by osteoclasts. During bone growth occurring during the first and second decades of life, bone formation precedes and exceeds bone resorption, and thus leads to an increase in the length and width of bone. Growth of the diaphyses lengthens the bone, at the same time the formation of new periosteal bone by the osteoblasts increases the diameter of the diaphyses; and endosteal resorption of bone by osteoclasts increases the diameter of the medullary cavity and thus makes the cortices thinner. Formation of new bone consists of the formation of osteoid tissue by the osteoblasts with mineralization occurring 13 d after the osteoid tissue has been formed (Buckwalter *et al.* 1995). Bone growth during this stage is important for the formation of a normal skeleton, and for the pelvic development in girls in preparation for childbirth. Approximately 90 % of adult bone mass accrues during the growth phase of bone, and after the growth in bone length stops, bone density and thickness continue to increase during early adulthood reaching its programmed maximum in the third decade (Bronner, 1994), after which the age-dependent decrease commences. Bone formation and resorption could be reflected by levels of enzymes from bone cells, by-products of bone matrix synthesis, or breakdown products of bone in blood or urine. These biochemical markers provide information about bone-turnover rate and are useful tools in studying the relationship between nutrient intakes and other factors, and bone health (Kleerekoper, 1996; Robins & New, 1997).

Methods of bone mass measurement

Non-invasive methods to assess bone mass in children and adolescents include single-photon absorptiometry (SPA), dual-photon absorptiometry (DPA), dual-energy X-ray absorptiometry

https://doi.org/10.1079/NRR200120 Published online by Cambridge University Press

(DXA), and quantitative computed tomography (QCT) (Slosman *et al*. 1995). SPA uses ^{125}I as the radioactive source and it is limited to the measurement of bone surrounded by thin soft-tissue with constant thickness, i.e. peripheral skeleton, especially the radius. DPA uses ^{153}Gd as the radioactive source and can measure bone surrounded by irregular thicknesses of soft-tissue, such as the spine. Further improvements in bone mass measurement have been made by the development of DXA, in which the radioactive sources are replaced by X-ray sources. Since there is no radioisotope source to decay, DXA has better precision and accuracy than DPA. QCT is also based on X-ray attenuation, and it can determine bone volumetric density by measuring an external standard at the same time. Two important characteristics of a technique are precision and accuracy. Precision, also called reproducibility, is the ability to make reproducible measurements without regard to accuracy. Accuracy is the ability to give a measured value for a reference bone or phantom that is close to its true value. When all these techniques are evaluated according to their precision, accuracy, cost-effectiveness and irradiation dose, SPA is the first choice for measuring the radius, and DXA is the first choice for measuring the spine and hip. QCT has the advantage of being able to assess the density of bone in the axial and appendicular skeleton without influence from body size. However, its high radiation dose and cost limit its use in children and adolescents (Ponder, 1995; Slosman *et al*. 1995). These limitations have been partially solved by the recent development of peripheral QCT scanners, which allow a high geometric resolution with minimal radiation exposure for the measurement of the appendicular skeleton in children (Gilsanz, 1998; Schönau, 1998). Quantitative ultrasound has recently been used to assess appendicular bone by measuring the speed of sound and ultrasound attenuation measurements (broad-band ultrasound attenuation). Quantitative ultrasound measurements have been mainly used in the calcaneus in adults. Ultrasound values are influenced by many structural variables, and the constantly changing macrostructure of children and adolescents make the data difficult to interpret in children (Gilsanz, 1998).

Bone mass measurements are usually expressed as either bone mineral content (BMC) or bone mineral density (BMD). BMC indicates the amount of bone mineral in a measured length of bone. The unit for BMC is g/cm in SPA and DPA, and is by convention g for DXA. The value of the ratio of BMC:projected area of the bone is areal BMD (g/cm^2). Since changes in bone thickness in growing children and adolescents may lead to an increase in areal BMD even though actual bone density may remain unchanged, some researchers have suggested the use of volumetric BMD, which is calculated on the basis of BMC and bone dimension values provided by DXA, with the assumption that the sites being measured (lumbar spine, femoral neck, and mid-femoral shaft) are cylinders (Cowell *et al*. 1995; Lu *et al*. 1996). Seeman (1997) has correctly pointed out that ‘density’ is a misleading term, and prefers the use of the term ‘bone mass’, or ‘apparent bone density’. However, the term BMD will be used in the present paper referring to areal BMD, unless otherwise stated, because this is the measurement produced by SPA, DPA, or DXA in most studies and thus most frequently used in the literature. Another reason for the general use of areal BMD is that areal BMD has been shown to relate to bone strength better than volumetric BMD because it reflects both the thickness and the integrated density of the skeletal piece (Riggs & Melton, 1988; Brinckmann *et al*. 1989). However, areal BMD data have to be interpreted with great caution, especially when making comparisons between different age, sex and ethnic groups (Seeman & Hopper, 1997), because of a lack of information about population variance in bone structure. Comparisons within groups produce more reliable conclusions, as must be borne in mind throughout this review.

Bone mass accumulation during adolescence

The pubertal growth spurt during adolescence brings about profound alterations in body dimensions and composition. Adolescence is the time of dramatic changes in height, weight and body composition, and at the same time is a crucial period for bone development.

Adolescence is the critical period for bone mass accumulation

Peak bone mass is the amount of bony tissue present at the termination of skeletal maturation. Although it is generally considered that peak bone mass is achieved at all sites in the mid-thirties (Ott, 1991; Recker *et al*. 1992), several recent studies focused on heterogeneity in bone mineralization among anatomical sites have suggested peak bone mass at certain sites may be attained before the end of the second decade (Turner *et al*. 1992; Young *et al*. 1995; Sabatier *et al*. 1996). Matkovic *et al*. (1990) studied twenty-four pairs of parents (mother and father) and daughters and showed that by 16 years of age girls had accumulated 90–97 % of the radial and spinal bone mass of their mothers.

However, there is no doubt that pubertal years are the critical years for bone mass accumulation, and factors affecting this period will affect peak bone mass attainment profoundly. Kröger *et al*. (1993) carried out a prospective study in sixty-five healthy Finnish children and adolescents aged 7–20 years. They found the annual increases of BMD and bone volumetric density in both spine and femoral neck were most marked in females at the time of menarche (during the age of 11–13 years), and in males between the ages of 13 to 17 years. During this period, the accumulation rate in areal BMD increased four- to sixfold in females and males respectively. Another study in 198 Swiss adolescents aged 9–19 years found that the increment rate in BMD:BMC was particularly pronounced over a 3-year period, from 11 to 14 years of age, and this increment fell dramatically after 16 years of age and/or 2 years after menarche (Theintz *et al*. 1992). The larger increase in bone size and cortical thickness in males than in females may be due to a more prolonged bone maturation period in males (Bonjour *et al*. 1994). Bonjour *et al*. (1991) assessed bone mass in 207 healthy Caucasian boys and girls aged 9–18 years in Switzerland. They found that bone mass at different skeletal sites continued to increase substantially between 15–18 years in males, whereas the increase in bone mass at the levels of both lumbar spine and femoral neck slowed down markedly from 15–16 years in female adolescents. There is an asynchrony between the gain in height and bone mass growth. In a 4-year study of 228 US children over a total age range of 9·5 to 19·5 years. Martin *et al*. (1997) reported that peak height velocity occurred at 13·3 years in boys and at 11·4 years in girls, but BMC velocity did not peak until 1·2 years after peak height velocity in boys and 1·6 years after peak height velocity in girls. After the growth in height stops, there is still a period during which the Ca content and amount of bone (bone mass) continue to increase. Peak BMC velocity was 320 g per year in boys and 240 g per year in girls, corresponding to a daily Ca retention of 282 mg in boys and 212 mg in girls. From these studies, we can see that there is a pronounced increase in accumulation rate of bone mass during pubertal development, and it occurs earlier in girls than boys due to the earlier onset of puberty in girls; boys attain higher peak bone mass resulting from their longer bone maturation period; skeletal mineralization continues beyond the age of maximum height in both sexes. Similar results were found for eighty-two white US girls by Lloyd *et al*. (1998); mean age for peak velocity and peak accumulation for total body BMC and BMD being 13·5 and 17·5 years respectively.

Bronner & Abrams (1998) recently published an analysis of Ca turnover in girls, by examining 100 dual-tracer stable-isotope studies of a total of sixty-eight US girls, aged 5–18 years.

https://doi.org/10.1079/NRR200120 Published online by Cambridge University Press

Table 1. Studies on factors affecting bone mass in childhood and adolescence

Reference	*n*	Nationality and gender	Age (years)	Bone mass measurement	Factors studied	Main outcomes
Boot *et al.* (1997)	500	Dutch Boys, girls	4–20	BMD of LS and TB by DXA	Weight, height, pubertal stage,CaI and PA	The major independent determinant of BMD was Tanner stage in girls and weight in boys. BMD was positively correlated with height in both sex, and CaI and PA in boys
Cadogan *et al.* (1998)	37	British Girls	12–14	Total-body BMC, total-body BMD, DXA	Weight, height, lean body mass, bone biomarkers, hormones, fractional Ca absorption	Lean mass gain and oestradiol most closely associated with bone mineral gain
Cheng *et al.* (1998)	179	Hong Kong Boys, girls	12–13	BMD of LS by DXA, BMC of DR by SPA	Weight, height, pubertal stage, physical activity, physical fitness, dietary intakes	Age, weight, pubertal stage, physical fitness and muscle strength significantly correlted with BMD and BMC. No association with Ca intake
Du (1998)	517	Chinese Girls	12–14	BMC of DR and midradius by SPA	Weight, height, bone age, Tanner stage, PA, CaI, milk and vitamin D intake	BMC was significantly correlated with all factors studied. Milk intake is the major dietary determinant of bone mineral status of those girls
Glastre *et al.* (1990)	135	French Boys, girls	1–15	BMD of LS by DXA	Weight, height, body surface, bone age, pubertal stage, CaI, and vitamin D supplementation	BMD was significantly correlated with all factors studied except CaI and vitamin D supplementation
Henderson *et al.* (1995)	115	Australian Girls	18	BMD of LS, PF, and DR by DXA	Mother's BMD, weight, blood and urine Ca, serum sex hormone, nutrient intakes, aerobic fitness, trunk muscle strength, and PA	BMD of all sites was significantly correlated with mother's BMD, weight, lean body weight, trunk strength, PA, and aerobic fitness. CaI was correlated with BMD at the trochanter site only. No association between BMD and serum Ca or sex hormone level
Ilich *et al.* (1996, 1998)	456	USA Girls	8–13	BMD of total body and DR, total body Ca	Skeletal age, height, weight, lean body mass, body fat	Bone area, lean body mass, body fat, skeletal age, dietary Ca
Jouanny *et al.* (1995)	183 and parents	French Boys, girls	> 15	BMD of TB by DXA	Parents' BMD, weight, height, BMI, CaI, and PA	BMD of children was significantly correlated with parents' BMD, weight, height, BMI in both sexes, and CaI and PA in boys
Katzman *et al.* (1991)	45	USA Girls	9–21	BMC and BMD of midradius by SPA, LS and TB by DXA and DPA, and FN by DXA	Weight, height, pubertal status, CaI and PA	BMC and BMD were correlated with weight, height, and pubertal stage. No significant association with CaI and PA

Lonzer *et al.* (1996)	28 and parents	USA Boys, girls	5–20	BMD of LS by DXA	Parents' BMD, weight, height, BMI, Tanner stage, CaI, and PA	BMD of children was positively correlated with all factors studied except CaI and PA
Rice *et al.* (1993)	35	Canadian Girls	14–18	BMC and BMD of TB and LS by DPA	Weight, height, body composition, sexual maturity, endocrine status, PA, strength, fitness	All BMC and BMD were significantly correlated with weight, growth hormone, and double-leg strength. Weight was the most important determinant
Ruiz *et al.* (1995)	151	French Boys, girls	7–15·3	BMD of LS and upper femur by DXA	Weight, height, sexual maturation, CaI and PA	BMD was correlated with all factors studied. Dietary Ca was main determinant of vertebral mineral density
Turner *et al.* (1992)	138	New Zealand Girls	16·4 (SD 0·34)	BMD at LS and hip by DPA	Weight, height, PA, and CaI	BMD showed positive correlation with all factors studied. Weight was the best predictor
Uusi-Rasi *et al.* (1997)	176	Finnish Girls	8–20	BMC and BMD at LS, FN and DR by DXA	Weight, body fat, height, muscle strength, sexual maturation, PA and CaI	BMC and BMD were associated with all factors studied except CaI. Weight and Tanner stage were the most important determinants
Young *et al.* (1995)	215 twin pairs	Australian Girls	10–26	BMD of TB, LS and hip by DXA	Weight, height, lean mass, fat mass, menarcheal history, CaI, and PA	BMD of lumbar and total body were associated with menarcheal status, weight, height, lean and fat major independent determinant of bone mass at the hip

BMD, bone mineral density; LS, lumbar spine; TB, total body; DXA, dual-energy X-ray absorptiometry; CaI, Ca intake; PA, physical activity; BMC, bone mineral content; DR, distal radius; SPA, single-photon absorptiometry; DPA, dual-photon absorptiometry; PF, proximal femur; FN, femoral neck.

They reported that bone Ca and deposition rates, the size of the exchangeable bone Ca compartment and intestinal Ca absorption all peaked at or near menarche. Since these authors found that bone Ca deposition increased 70 % faster than Ca absorption, they considered that this was not in response to increased intestinal absorption but was probably in response to developmental signals.

Factors affecting bone mass accumulation in adolescence

Recent studies about factors affecting bone mass accumulation in childhood and adolescence, including unpublished data on Chinese adolescent girls from the cross-sectional study carried out by Du (1998) in our department, are summarized in Table 1. Bone mass is determined by racial and genetic factors, body weight, physical activity, nutritional intake (include Ca), and endocrine factors (Heaney, 1986; Smith, 1993). Although interactions among these factors are complex, there is a large body of evidence indicating that genetic factors are probably the greatest influence on achievable bone mass (Smith *et al*. 1973; Pocock *et al*. 1987), but bone mass may be modified by other factors, especially environmental factors, and it is these factors which are susceptible to intervention to improve bone health. The modifiable factors of diet and physical activity are notoriously difficult to measure reliably, and this may explain the varying results obtained in different studies.

Genetic factors

Heredity and racial factors are the most important determinants of peak bone density. Bone mass differs from one race to another. Trotter *et al*. (1960) found from a study of skeletons of forty white and forty black Americans that blacks had 10–14 % higher volumetric densities. A review (Pollitzer & Anderson, 1989) based on ninety references concluded that blacks have higher bone density than Caucasians and Asians, while Nelson & Barondess (1997) showed that US children of Chaldean (Middle Eastern) descent had whole-body BMC similar to that of blacks and significantly higher than that of white children. A study using QCT compared results for healthy and osteoporotic Japanese men and women with data for Caucasian US subjects and indicated that the Japanese appeared to have lower trabecular bone density (Fujii *et al*. 1989).

Gilsanz *et al*. (1991) used QCT to measure vertebral bone density in a cross-sectional study of seventy-five black and seventy-five white US females aged 2–20 years. Subjects were matched for age and stage of sexual development. Vertebral bone density did not differ between black girls and white girls before puberty, but the magnitude of pubertal increase was much greater in black than white girls (34 % *v.* 11 %). A longitudinal study of 700 US children aged 8–10 years showed that black children had significantly higher whole-body BMC, BMD, and lean mass than whites and the annual change in whole-body BMC, BMD and lean mass were also greater in the black children (Nelson *et al*. 1997). Further, Slemenda *et al*. (1997) found that black children had higher BMD and lower serum concentrations of osteocalcin and tartrate-resistant acid phosphatase, reflecting reduced rates of bone turnover, than white children. Thus black–white differences in bone mass appear to be related to greater lean mass and lower bone turnover rate in blacks.

Within races, twins and parent–offspring models have suggested that 46–80 % variance in BMD as well as other bone mass measures, including bone area and estimated volumetric

https://doi.org/10.1079/NRR200120 Published online by Cambridge University Press

BMD could be explained by inherited factors (Pocock *et al*. 1987; Krall & Dawson-Hughes, 1993; Morrison *et al*. 1994; Ferrari *et al*. 1998). Family-resemblance studies have suggested that bone mass of children and adolescents is strongly influenced by genetic information from both mothers and fathers (Matkovic *et al*. 1990; Guéguen *et al*. 1995; Jouanny *et al*. 1995; Lonzer *et al*. 1996). Seeman *et al*. (1989) demonstrated that premenopausal daughters of women with osteoporosis had reduced bone mass of the lumbar spine and perhaps of the femoral neck, probably putting them at increased risk of fractures. These authors considered that their data pointed to low peak bone mass rather than programmed excessive bone loss as a determinant of post-menopausal osteoporosis. O'Brien *et al*. (1998) compared girls and women from osteoporotic families with those from non-osteoporotic families and found that the former had higher rates of bone turnover.

Results of twin studies have provided further evidence that genotype plays a major role in the determination of bone density. Smith *et al*. (1973) measured radial bone mass in seventy-one juvenile and eighty adult US twin pairs of both sexes and found significantly larger variation in intrapair differences in dizygotic than monozygotic twins. Pocock *et al*. (1987) showed that BMD was significantly more highly correlated in monozygotic than in dizygotic twins also for the spine and proximal femur in Australian male and female premenopausal twin pairs, and confirmed the findings of Smith *et al*. (1973) for the forearm. While most studies have suggested that genetic factors operate on both weight-bearing and non-weight-bearing bone in the same way, some studies (Dequeker *et al*. 1987; Pocock *et al*. 1987) have stated that genetic determinants appear to count somewhat more in the development years in the vertebrae, whereas environmental factors appear more significant for the radius.

The mechanism of the genetic influence on bone density remains poorly understood. Recently, a number of studies have examined the relationship between BMD and allelic variation in the vitamin D receptor (VDR) gene (Morrison *et al*. 1994; Fleet *et al*. 1995; Spector *et al*. 1995; Gross *et al*. 1996; Salamone *et al*. 1996; Tokita *et al*. 1996; Arai *et al*. 1997) at sites cleaved by *Bsm*I, *Apa*I, *Tak*I and *Fok*I endonucleases. Morrison *et al*. (1994) from their study of 250 normal healthy Caucasian Australian twins demonstrated that bb genotype (*Bsm*I) was associated with higher bone density at the lumbar spine and femoral neck than the BB genotype with Bb intermediate. However, other studies have shown the reverse (Salamone *et al*. 1996) while others found no effect (Guarnero *et al*. 1996; Uitterlinden *et al*. 1996; Kristinsson *et al*. 1998).

Research results in children have also been controversial. Sainz *et al*. (1997) stated that VDR gene alleles predict bone density (measured by QCT) in pre-pubertal US girls of Mexican descent since girls with aa and bb genotypes had 2–3 % higher femoral bone density and 8–10 % higher vertebral bone density than girls with AA and BB genotypes. However, Baroncelli *et al*. (1999) did not find any difference in radial BMD, lumbar BMD and estimated volumetric lumbar BMD among VDR genotypes at *Bsm*I sites in a group of healthy Caucasian Italian girls. Ames *et al*. (1999) studied seventy-two US children and found that the *Fok*I polymorphism at the VDR translation initiation site was significantly associated with BMD and Ca absorption using dual-tracer stable-isotope techniques. Ca absorption of FF homozygotes was 41·5 % greater than that of ff homozygotes and 17 % greater than that of Ff heterozygotes. Total BMD was 8·2 % greater in the FF genotype than the ff genotype, and 4·8 % greater than the Ff genotype, implying that the VDR genotype influenced BMD through regulation of Ca absorption.

However, in a study of 195 women within 5 years after menopause, it was found that the changes in bone density occurring with age were not related to VDR genotype, suggesting that the relationship between VDR alleles and bone density mainly result from the effect of the VDR gene on determination of peak bone mass (Keen *et al*. 1995). This suggestion was supported by the findings of Riggs *et al*. (1995) who found that age modulated the effects of VDR

genotypes on femoral neck BMD, and the effect of genotype was greatest among younger women and declined with age, culminating in no discernible difference by the age of 70 years. Since bone density is related to body size, body mass, and hormone levels, all of which are also under genetic control, genetic factors that influence skeletal growth and pubertal development may also influence peak bone density.

Some recent studies suggest that gene–nutrient interactions may influence the importance of the effect of Ca on bone mass. Dawson-Hughes *et al*. (1995) reported that women with BB allelic variants of the VDR had reduced Ca absorption efficiency on low Ca intakes in comparison with women with the bb variants. The response of lumbar spine BMD in seventy-two elderly male and female Swiss subjects to Ca intake was also reported to be related to VDR genotype with a positive association between Ca intake and change in BMD in heterozygotes (Bb) (Ferrari *et al*. 1995).

Other candidate genes which have been shown to be related to bone mass and/or strength include the oestrogen receptor gene (Kobayashi *et al*. 1996; Ongphiphadhanakul *et al*. 1998) and collagen type I-α-1 gene (Weichetova *et al*. 2000). Lorentzon *et al*. (1999) reported that the xx allelic variant of the oestrogen receptor gene was associated with a higher estimated volumetric spine BMD than the Xx allelic variant (361 *v*. 340 mg/cm^3, $P = 0{\cdot}04$) in a group of ninety Caucasian boys aged 16.9 years and *Xba*1 genotype predicted total body BMD, head BMD and estimated volumetric spine BMD. The potential insulin-like grown factor-I gene microsatellite polymorphism has shown no association with BMD in two studies, one of Caucasian premenopausal sister pairs (Takacs *et al*. 1999) and the other of Japanese postmenopausal women (Miyao *et al*. 1998).

The influence of genotype and interaction with nutrition appears to be an area requiring far more investigation, and this is particularly the case for children and adolescents.

Body weight, height and composition

Cross-sectional and prospective studies have shown a positive relationship between height, weight and bone mass in children and adolescents, with body weight appearing to be the most important determinant among modifiable factors in this group (Turner *et al*. 1992; Moro *et al*. 1996; Boot *et al*. 1997; Uusi-Rasi *et al*. 1997). Although in adults of both sexes height is not a determinant of bone mass, a significant positive association in children and adolescents has been reported, at least up to 15 years of age (Glastre *et al*. 1990). In a study of 207 9–18-year-old healthy Caucasian boys and girls in Switzerland, Bonjour *et al*. (1991) showed that there was close correlation between height and BMC in females up to about 1·55 m (average age 13·0 years) and in males up to 1·60 m (average age 13·5 years). Above these mean height values, the association disappeared in females and became much weaker in males. However, *r* values were not given in this study. The diminution of the association between height and bone mass after a certain age may result from the asynchrony between the gain in height and bone mass during growth. The stronger correlation between bone mineral and body mass rather than body height shown by this study suggests that the relationship between body weight and bone mass does not simply result from an increase in body size, but may be related to increased mechanical loading on weight-bearing bones as a result of the increasing body mass during growth. The positive correlation between body weight and BMD may result from the mechanical load of body mass. In addition, the increase in lean body mass associated with greater body weight contributes directly to skeletal loading through muscle mass load on the skeleton, and increased fat mass supports greater conversion of adrenal androgens to oestrogens. Another

explanation is that low body weight may result from inadequate intake of nutrients generally (including those, such as Ca, closely associated with bone health), and thus is associated indirectly with poor bone mass.

Rice *et al*. (1993) studied the correlates and determinants of BMC and BMD in healthy Canadian girls aged 14–18 years and found that the correlation between body mass and the measures of BMC was higher than between body mass and BMD. This may reflect the effect of growth-related bone expansion on bone mineral status during this period. This finding is in accordance with other studies that have shown the positive correlations between BMC, BMD and body weight diminish or disappear when bone mineral measures are corrected for estimates of bone volume (Katzman *et al*. 1991; Kröger *et al*. 1992). Katzman *et al*. (1991) found a positive correlation between BMC, BMD, and bone mineral apparent density (BMC normalised to a derived bone reference volume) of lumbar spine, whole body, and femoral neck and age, weight, height, and pubertal stage in forty-five pre-pubertal and pubertal American girls. The highest correlations were obtained with BMC, intermediate correlations were seen with BMD, and the correlations with bone mineral apparent density were only modest or without significance. This difference may reflect that the pubertal increase in bone mineral may be attributable to bone expansion rather than to an increase in bone mineral per unit volume.

Although body weight is a powerful determinant of bone mass, excessive weight gain is not a desirable solution for osteoporosis prevention, because it may increase the risk of CHD, hypertension, diabetes and many other chronic diseases. Therefore, studies of body composition and bone mass become more important. DXA makes body-composition studies in obese and normal children and adolescents available by allowing a direct and accurate measurement of three body compartments: fat, lean, and BMC. A number of studies in children and adolescents have suggested that lean body mass has a stronger correlation with BMD than fat body mass (Young *et al*. 1995; Manzoni *et al*. 1996; Moro *et al*. 1996; Ilich *et al*. 1998; Cadogan *et al*. 1998). As mentioned earlier, lean mass can affect bone density by mechanical loading, and muscle mass loads the skeleton directly. The positive association between lean mass and bone mass includes the fact that muscle and bone may increase in response to the same types of external stimuli, such as physical activity, and by the same internal stimuli, such as growth hormone, insulin, and androgens (Slemenda, 1995). It seems that processes that favour the acquisition of lean body mass rather than fat mass, such as physical activity, should be encouraged to promote bone health in childhood and adolescence.

Endocrine factors

Hormonal factors play an important role in bone mass acquisition in children and adolescents. During adolescence, growth hormone as well as sex hormone levels increase, and both have a positive influence on BMD (Slootweg, 1993; Albertsson-Wikland *et al*. 1994). Ito *et al*. (1995) examined reproductive factors and BMD in 192 premenopausal and 327 post-menopausal Japanese women. Length of reproductive period had the strongest correlation with BMD, but early menarche was also significantly associated with BMD in both groups of women. Earlier menarche was also shown to favour BMD in adolescent Japanese girls (Takahashi *et al*. 1996). This may be because early menarche is associated with earlier onset and longer duration of puberty (Marti-Henneberg & Vizmanos, 1997) providing the opportunity for more bone mass accumulation; however, direct evidence is not available. Finkelstein *et al*. (1992) compared radial BMD and spinal BMD of twenty-three men who had a history of delayed puberty with those of twenty-one men who underwent normal puberty in the USA. They found that adult

Table 2. Effects of calcium supplementation on bone measures in children and adolescents

Reference	Country	Sex	*n*	Age (years)	Ca (mg/d) Diet	Ca (mg/d) Supplement	Duration (months)	Effects on bone: Forearm	Femur	Lumbar spine	Total body
Andon *et al.* (1994)	USA	F	248	11	884	500/1000	6	–	–	–	+/+*
Bonjour *et al.* (1997)	Switzerland	F	149	8	880	850	12	+	+	0	–
Dibba *et al.* (2000)	The Gambia	M,F	160	10	342	1014	12	+	–	–	–
Johnston *et al.* (1992)	USA	M,F	90	10	900	718	36	+†	+†	+†	–
Lee *et al.* (1994b)	China	M,F	162	7	280	300	18	+	–	–	–
Lee *et al.* (1995a)	Hong Kong	M,F	84	7	570	300	18	+ NS	–	+	–
Lloyd *et al.* (1993)‡	USA	F	94	12	960	354	18	–	–	+	+
Lloyd *et al.* (1996a)‡	USA	F	91	12	983	360	24	–	+	+	+
Matkovic *et al.* (1990)	USA	F	12	14	800	890	24	+ NS	–	0	–
Nowson *et al.* (1997)	Australia	F	42 twin pairs	14	730	1000	18	–	+	+	–

F, female; M, male; +, positive effect; –, not measured; 0, no effect.
* Values are differences in gain for 500 and 1000 mg/day supplement respectively.
† Data in table are limited to prepubertal subjects (twenty-two pairs).
‡ Same study, follow-up at 18 and 24 months.

men with a history of delayed puberty had decreased radial and spinal BMD, and suggested that the time of puberty is an important determinant of peak bone density in men.

A number of cross-sectional studies of adolescents have shown a positive correlation between pubertal stage and BMD (Glastre *et al*. 1990; Katzman *et al*. 1991; Rico *et al*. 1993; Rubin *et al*. 1993; Ruiz *et al*. 1995; Lloyd *et al*. 1996*a*; Lonzer *et al*. 1996; Boot *et al*. 1997; Uusi-Rasi *et al*. 1997). The study by Rubin *et al*. (1993) of 299 white girls aged 6–18 years showed that up to 80 % variation in axial BMD was explained by weight and pubertal stage, with pubertal stage the strongest single predictor. Girls with above-median Tanner scores had higher bone acquisition rates in a Ca supplementation study by Lloyd *et al*. (1996*a*) (see Table 2 for details) compared with unsupplemented controls at the same stage. Conversely, in girls with below-median Tanner scores bone acquisition was not affected by Ca supplementation or dietary Ca level. Puberty has a greater influence on BMD in girls than in boys. Girls who had an early menarche or regular periods had higher BMD suggesting that oestrogen is an important determinant of BMD in girls during puberty (Boot *et al*. 1997). Oestrogen has known effects on bone mass. It can decrease the rate of the bone turnover, and inhibit the osteoclastic resorption of bone by affecting bone cell differentiation and function. It also has effects on parathyroid hormone and vitamin D metabolism. Lower levels of oestrogen may adversely affect bone mass (Lindsay *et al*. 1978; Saggese *et al*. 1997). A study carried out in forty-three white girls aged 13–20 years in the USA reported that oestrogen score (based on physiological events known to reflect circulating oestrogen levels) and testosterone levels were highly correlated with BMD of

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the spine, wrist, and foot. At maturity, those subjects with the lowest oestrogen exposure scores had lower BMD values for spine, wrist and foot (Dhuper *et al*. 1990). A longitudinal study of thirty-seven Caucasian British girls aged 12 years at study entry showed that oestradiol was strongly and significantly associated ($P<0{\cdot}01$) with bone gain over the 18 months of study. The authors concluded that oestradiol was a most important determinant of bone gain in pubertal girls and was probably responsible for the reduced bone turnover they observed in late puberty (Cadogan *et al*. 1998). Androgens may also be important determinants of bone density. Both trabecular and cortical bone mass development are related to androgen levels (Leuenberger *et al*. 1989). It was found that young US women with excess androgens had higher bone density than normal women (Buchanan *et al*. 1988). This suggests that androgens play an independent role in the determination of bone mass. How androgens influence bone density is not known, but may involve mechanisms similar to oestrogen because androgens can be converted to oestrogen in adipose tissue. Both oestrogen and androgen receptors have been demonstrated in bone cells (Saggese *et al*. 1997). Moreover, the chronology of the secretion of sex hormone also plays a role in bone development.

Ilich *et al*. (1997) appear to have carried out the most complete study available on the hormone 1α,25-dihydroxycholecalciferol ($1,25(OH)_2D$), derived from vitamin D, and bone mass in pubertal girls. In a cross-sectional study of a convenience sample of 178 girls aged 8–18 years, levels of $1,25(OH)_2D$ were found to peak at sexual maturity stages 3–4, age 11–13 years. This correlated to peak skeletal Ca accretion (g per year) and bone mass accumulation in total body and forearm, at those stages, as determined cross-sectionally. A subsample of fifty-seven girls who were pre-pubertal and at sexual maturity index 2 at baseline were followed up 1 year later. Baseline $1,25(OH)_2D$ levels in these girls were positively associated with change in total body BMC and BMD, and in radial BMC and BMD over 1 year. Dietary vitamin D was negatively associated with $1,25(OH)_2D$ levels at baseline; $1,25(OH)_2D$ levels were (just) significantly associated ($P=0{\cdot}0514$) with osteocalcin levels when measured at follow up. The authors concluded that: 'calcitriol [$1,25(OH)_2D$] is a significant correlate of bone mass accumulation during pubertal growth, presumably in response to the high requirements for Ca during this critical phase of skeletal development'. These observations are compatible with the concept that $1,25(OH)_2D$ production is controlled by the physiological process of growth and, except in vitamin D deficiency, is independent of vitamin D status.

Physical activity

The importance of physical activity in the development of peak bone mass is widely accepted. Most cross-sectional studies in normal children and adolescents (as opposed to comparisons of athletes *v*. non-athletes) have shown a positive association between current physical activity level and BMD (Slemenda *et al*. 1991; Turner *et al*. 1992; Rubin *et al*. 1993; Boot *et al*. 1997; Uusi-Rasi *et al*. 1997), while some failed to show any relationship (Katzman *et al*. 1991; Rice *et al*. 1993; Lonzer *et al*. 1996; Kardinaal *et al*. 2000). This discrepancy between studies may be due to differences in methods of assessment of activity level (difficult to measure reliably), statistical procedures, and small sample sizes. The study by Kardinaal *et al*. (2000) was large (a random sample of 1116 Caucasian girls aged 11–15 years from six European countries), but it is not evident that possible inter-country differences in bone structure were taken into account. Prospective studies have indicated that physically active subjects have significantly higher mineralization rates than sedentary subjects (Slemenda *et al*. 1994; Bennell *et al*. 1997; Taaffe *et al*. 1997). A study of the role of physical activity and sunlight exposure in bone mass measures of a cohort of 8-year-

old children in Tasmania (Jones & Dwyer, 1998) demonstrated that for boys, sports participation had the strongest association with BMD, with sports participants having 4·2 % higher BMD at the femoral neck and 4·3 % higher BMD at the spine than those who did not participate in organised sport. For the girls in the study PWC170 (bicycle ergometric physical work capacity at a pulse of 170 beats/min) was the factor most strongly associated with BMD at both femur and spine; girls in the highest quartile of PWC170 had size-adjusted BMD 7·2 % higher at the femoral neck and 5·1 % higher at the spine compared with those in the lowest quartile.

The ability of bone to adapt to mechanical loading is much greater during growth, and particularly during adolescence, than after maturity (Parfitt, 1994); however, the physiological mechanisms by which mechanical loading affects bone mass are not very clear, especially the transmission of the signal (bone stress) to the cells responsible for remodelling bone. The mechanical variables related to the initiation of the remodelling response are most likely to be strain energy density, longitudinal shear stress, and tensile stress-strain (Brown *et al*. 1990). Brighton *et al*. (1991) showed *in vitro* that cyclical biaxial strains exerted at particular levels on isolated rat calvarial bone cells in cell culture increased cell proliferation and decreased macromolecular synthesis, probably due to an increase in prostaglandin E_2. However, the effects of loading on intact living bone are more complex because cells exist within a matrix.

The effects of mechanical loading on BMD are highly site-specific as shown in tennis players, whose BMD of dominant arms, but not non-dominant arms, had significantly higher value than that of controls (Kannus *et al*. 1995). It appears that moderate regular exercise maintains bone mass and that more vigorous regular exercise increases bone mass in children and young adults. Forceful and rapid muscular movement, such as gymnastics and fast ball games can benefit bone mass gain, whereas repetitive movements with moderate power and speed, such as running and swimming have little influence on bone density. Grimston *et al*. (1993) reported that children involved in weight-bearing sports producing significant impact loading on the skeleton had greater femoral neck bone density and a trend for greater spinal bone density, than children in competitive swimming producing loads to bone primarily through muscular contraction. These findings can be explained by Lanyon's concept: bone mass is maintained at appropriate levels to afford structural competence of functional loading (Lanyon, 1986, 1987). Mechanical loading of the skeleton during physical activity causes a strain or deformation of the bone. This strain becomes an osteogenic stimulus if it is greater than the optimum strain determined for that area, thus leading to an increase in bone mass. Unlike Ca intake, which has a threshold on its influence on bone mass, physical activity does not have such a threshold, i.e. the greater the mechanical loading on the bone, the higher the mass of that bone. One concern related to physical activity and bone mass is that intensive and endurance training may lead to oestrogen deficiency and eating disorders which are associated with bone loss. However, this risk is confined to sports requiring very hard endurance training, such as running, or favouring low body weight, such as ballet dancing (Prior *et al*. 1990; Bonen, 1992; Hetland *et al*. 1993). Since the response of bone to cyclical loading may decline with age, the possibility of increasing bone mass by physical activity wanes after puberty (Gunnes & Lehmann, 1996). Moreover, the high BMD induced by physical activity is only maintained if the activity level is sustained.

Nutritional factors

Study of nutritional factors needs reliable food composition data and validated dietary methods. Reliable food composition data for bone nutrients have become more available in recent times, but often dietary methods (particularly food frequency questionnaires) have not been validated,

https://doi.org/10.1079/NRR200120 Published online by Cambridge University Press

and if they have, the method of validation does not appear in the literature. Dietary prevalence studies must therefore be interpreted with caution.

Calcium. Ca intake is an important determinant of peak bone mass among young adults by influencing skeletal Ca retention during bone growth. Although most cross-sectional studies have found a positive correlation between dietary Ca and BMD during childhood and adolescence (Sentipal *et al*. 1991; Fehily *et al*. 1992; Turner *et al*. 1992; Rubin *et al*. 1993; Gunnes, 1994; Ruiz *et al*. 1995; Pettifor & Moodley, 1997; Ilich *et al*. 1998), others have found little association (Kristinsson *et al*. 1998; Kardinaal *et al*. 1999, 2000) or none (Glastre *et al*. 1990; Katzman *et al*. 1991; Lonzer *et al*. 1996; Uusi-Rasi *et al*. 1997; Bonofiglio *et al*. 2000). A threshold effect for Ca intake may explain some of the inconsistencies. Below the level of threshold intake, increase in Ca intake will benefit skeletal accumulation, whereas above this level, further increase in intake will not result in further skeletal retention. The threshold intake level for ages 9–17 years was reported to be 1500 mg/d (Matkovic & Heaney, 1992).

A detailed balance study of Ca retention of adolescent females at different levels of Ca intake (841–2173 mg/d) by Jackman *et al*. (1997) supported the idea that Ca retention plateaus at a certain level of intake. They found that maximal Ca retention was 473 mg Ca/d (95 % CI 245, 701), and that at higher post-menarcheal ages maximal Ca retention was lower but the intake required to achieve this was not affected. The minimal intake to achieve maximal retention in some subjects was 1300 mg/d and retention continued to improve with intakes >2 g/d.

Vitamin D. Vitamin D is important for bone mass accumulation during childhood and adolescence because it is an essential factor for Ca absorption, bone mineralization and Ca homoeostasis. Vitamin D can either be acquired from the diet or be synthesized in the skin from 7-dehydrocholesterol in a reaction catalysed by u.v. irradiation. Although both sources need to be taken into account, the definitive test of vitamin D status is measurement of plasma 25-hydroxycholecalciferol (25(OH)D). However, in many populations vitamin D status is determined mainly by the extent of exposure to solar u.v. light rather than dietary intake.

Cross-sectional studies by Du (1998) demonstrated that among 1248 Beijing, China, adolescent girls aged 12–14 years, clinical vitamin D deficiency was present in 9 % during winter, while subclinical vitamin D deficiency rates were 45 % in winter; with improvement in summer when subclinical vitamin D deficiency rates were reduced to about 7 %. When 228 of the girls for whom paired winter–summer data were available were examined on the basis of 25(OH)D levels, it was found that those (6·1 %) with low levels (<12·5 nmol/l) in winter and summer had the lowest BMC at distal ulna, while those (41·7 %) with normal levels had the highest BMC, and those (52·2 %) with low levels in winter only had intermediate BMC, although results were not significant when corrected for age, bone width, bone age, height, body weight and milk intake, possibly due to the small number of girls for whom complete data were available (Du *et al*. 1999). Kristinsson *et al*. (1998) investigated 259 young women aged 16–20 years in Reykjavik, Iceland, at 64°N. While they did find that 18·5 % of subjects had serum 25(OH)D levels <25 nmol/l, no significant association was found with BMD measured at lumbar spine, hip, radius and total skeleton in the older women, while there was a significant association with total forearm BMC and BMD in the 16-year-olds in both univariate and multivariate analyses. It would have been interesting if this study had included younger subjects. The largest study located (Kardinaal *et al*. 1999) found no association between vitamin D status and radial BMD of adolescent girls in Europe.

Few studies have investigated dietary vitamin D intake and bone mineral status of children and adolescents. Du *et al*. (1999) found a positive correlation between measured oral vitamin D intake and BMC of 517 Beijing 12–14-year-old girls. Unfortunately no compositional data were available for the vitamin D content of Chinese foods, and British food composition data were used for this nutrient, apart from fortified foods. In this study, vitamin D intake was not significant for inclusion in the multiple regression model of forearm BMC. Glastre *et al*. (1990) reported no significant difference in BMD when comparing 1–15-year-old Caucasian French children with and without vitamin D supplementation; however, neither vitamin D supplementation dose nor duration were recorded in this study.

So far as sunlight exposure is concerned, Gunnes & Lehmann (1995,1996) found no association in Norwegian children and adolescents, aged 8–16 years, between hours of daylight exposure and BMD of forearm when studied cross-sectionally at baseline, nor after follow-up 1 year later. However, Jones & Dwyer (1998) measured (usual) sunlight exposure using a questionnaire previously validated against polysulfone badges (which measures u.v. B exposure) in a cohort of Tasmanian 8-year-olds. Their results showed a weak but not significant association between winter sunlight and BMD at femoral neck and lumbar spine in boys, whereas for girls the association was stronger and statistically significant. By contrast, summer sunlight exposure showed no association with BMD at either site for boys or girls. Plasma vitamin 25(OH)D levels were not measured in this study. It is interesting to note in this context that seventy-five older girls in the Icelandic study by Kristinsson *et al*. (1998), who had been exposed to artificial u.v. from sun lamps (dose not stated) in the previous 3 months had serum 25(OH)D levels 55 % higher ($P<0{\cdot}01$) than those who were not so exposed. In Du's (1998) study of 12–14-year-old girls in Beijing, despite their lower doses of measured u.v. B exposure (and lower plasma vitamin D levels) compared with rural girls, the urban girls had higher BMD. For example, BMD at distal one-third radius was 0·597 (SD 0·062) g/cm^2 in rural girls, and 0·6512 (SD 0·120) g/cm^2 in urban girls, while u.v. B exposure was significantly higher ($P<0{\cdot}05$) in both winter and summer in rural girls (34·2 (SD 11·4) and 59·8 (SD 13·5) mJ/cm^2 respectively) than in urban girls (14·9 (SD 5·1) and 36·0 (SD 11·4) mJ/cm^2 respectively).

It would seem that there is no definitive evidence associating increased daylight exposure with improved bone density in children or adolescents, probably because of the poverty of the methods of measurement, or the failure to measure all relevant factors thoroughly. Definitive studies of the effects of vitamin D on bone density in childhood and adolescence would ideally need reliable data for dietary assessment (including supplementation), for vitamin D in local foods, for u.v. B exposure, for true skin conversion of 7-dehydrocholesterol using the ampoule method (Webb *et al*. 1988) and for serum 25(OH)D levels.

Protein. The role of protein in Ca metabolism has been most investigated in adults. The study by Teegarden *et al*. (1998) of 215 young US women aged 18–31 years showed that protein was an important factor for BMD in multivariate analysis. In a study of women aged over 50 years, Kerstetter *et al*. (2000) showed a significant association ($P=0{\cdot}037$) between low protein intake and low bone density, after adjusting for age and body weight, and also when the analyses were restricted to subjects with Ca intakes ≥800 mg/d, when examining data for 1822 non-hispanic white US women who participated in the National Health and Nutrition Education Survey (NHANES) III. However, Cooper *et al*. (1996) showed a positive relationship between protein intake and BMD only in the seventy-two premenopausal US women aged 30–50 years studied and not in post-menopausal women.

Since bone comprises a protein matrix in which bone minerals are laid down, protein would seem to be important for bone growth and development in childhood and adolescence.

Conigrave *et al*. (2000) have demonstrated that the extracellular Ca-sensing receptor is activated also by amino acids, especially L-phenylalanine and L-tryptophan, raising the speculation that dietary protein may favour Ca uptake.

Nevertheless, epidemiological evidence associating protein with bone mass in children and adolescents is lacking. Although circumstantial evidence of protein deficiency's involvement in nutritional rickets has been provided by Lulseged & Fitwi (1999), the most complete study appears to be that of the Chinese girls studied by Du (1998) who, using Chinese food composition data and a validated dietary method (Du *et al*. 1997*b*), demonstrated a high prevalence of underweight at 32 % (Du *et al*. 1998) and had low protein intakes at 50 (SD 9) g/d. While higher BMD was associated in univariate analyses with higher intakes of Ca, protein, vitamin D, P and milk, there was no association between protein intake and bone mineral status, when multivariate analysis was performed on all factors (Du *et al*. 1999).

Other dietary factors. Other dietary factors that may affect BMD include dietary fat, fibre, vitamin C, Zn, Mg, K, Na and vitamin K (New *et al*. 1997), and fruits and vegetables (Tucker *et al*. 1999; New *et al*. 2000); however, these have not been so intensively investigated in adolescents as in adults. Gunnes & Lehmann (1995) reported that, in Norwegian children and adolescents, in addition to Ca intake, the current intake of saturated fat, fibre, and vitamin C were also positively associated with forearm BMD. In the same study group 1 year later these authors found BMD accretion was positively correlated with the intake of polyunsaturated fat and negatively correlated with Na intake (Gunnes & Lehmann, 1996). In young women aged 18–31 years, Teegarden *et al*. (1998) identified P intake as an important dietary determinant of BMD and BMC along with protein and Ca using multivariate analysis.

Milk intake. Several retrospective studies (Sandler *et al*. 1985; Stracke *et al*. 1993; Murphy *et al*. 1994; Renner, 1994; New *et al*. 1997; Teegarden *et al*. 1999) have shown positive effects of milk consumption during childhood and adolescence on BMD later in life while others have not (Ulrich *et al*. 1996). However, of particular interest is a randomised controlled trial of free school milk directed to 581 Welsh schoolchildren aged 7–8 years, and identified as at risk of poor growth by virtue of socio-economic status, carried out by Baker *et al*. (1980). The supplemented children received 190 ml regular milk (228 mg Ca) per school day over six terms (21·5 months). Fehily *et al*. (1992) were able to follow up 371 supplemented and unsupplemented children 14 years later, and established no differences between them in terms of current intakes of Ca, and energy and in terms of milk consumption. BMC and BMD were slightly higher among those who had received the milk supplement; however, these results were not statistically significant. It would have been surprising indeed if such a short-term supplementation had produced any sustained effects on bone mass, which was not measured in the original study (Baker *et al*. 1980) but in the absence of baseline measures the study is difficult to interpret. It cannot be assumed in the absence of evidence, that bone mineral measurements were the same in both groups at baseline. The study by Du *et al*. (1998) of 517 girls in Beijing aged 12–14 years, for whom complete data were available, revealed by multiple regression analysis that the intake of milk and its products (independent of protein, Ca or vitamin D intake) was the dietary factor most favouring bone mineral content in this group.

The converse is true for children who avoid milk and its products according to Infante & Tormo (2000) who examined thirty patients aged 2–14 years, who for reasons of lactose or milk intolerance, short bowel syndrome or hypercholesterolaemia avoided milk consumption but were following various substitute dietary regimens. Fifty percent of the patients had

https://doi.org/10.1079/NRR200120 Published online by Cambridge University Press

osteoporosis or osteopenia as defined by BMD assessment. There was a highly significant correlation between the percentage Ca derived from milk or substitute and BMD.

Intervention trials for promoting bone health in childhood and adolescence

From studies about factors determining bone mass in childhood and adolescence, we can see that children and adolescents may be at risk of not obtaining optimal peak bone mass and developing osteoporosis later in life because of low body weight (lean body mass), sex hormone deficiency, inactivity, low u.v. exposure, low vitamin D status and low Ca intake. Will intervention to correct these risk factors improve bone health of children and adolescents and help to optimize their peak bone mass? Will ensuring the attainment of genetic potential for peak bone mass reduces the risk of osteoporosis in later life? Riggs & Melton (1992) after reviewing the range of treatment options for osteoporosis state that: 'Prevention is the only cost-effective approach to osteoporosis'. Longitudinal intervention trials provide useful information for testing this hypothesis by controlling confounding variables. They also provide very good evidence of the causal potential of the intervention factors used, and these can usually be measured and recorded reliably. It is important, however, in an intervention trial to measure any changes in the environment consequent to the intervention. For example, in a dietary or exercise trial, does the normal pattern of diet and exercise remain undisturbed, or does it change? This needs to be monitored using methods that are almost universally less reliable than measurement of the intervention factor itself. Unfortunately, intervention trials may be difficult to carry out double-blind. Ca supplements can use placebo controls, and milk- or Ca-fortified products can be made that are indistinguishable from regular products. However, exercise interventions and food supplements cannot be concealed, leading to problems with study design.

Anderson & Metz (1993) have strongly supported intervention strategies prior to puberty for females, with the goal of enabling all young females to achieve peak bone mass and density of the spine, hips and other bones prior to age 20 years. They also suggest that strategies for gaining 3–5 % additional bone mass in the third decade would prolong the time before the fracture threshold range is reached post-menopause.

Exercise intervention trials

Exercise intervention trials have shown that physical activity had a positive effect on bone mass accumulation in children and young adults (Snow-Harter *et al*. 1992; Morris *et al*. 1997). The mineralization rates were 1–11 % higher in exercise groups than controls, and the increase in increment rate was more pronounced in pre-pubertal girls. Especially worthy of mention is that in Snow-Harter's study in young women whose pubertal growth was already complete, the BMD of the controls did not change during the 8 months, whereas the runners and weight trainers had 1·3 % and 1·2 % gain in BMD respectively. This indicates that physical activity favours the increase of bone mass even after most of the bone mass has already been acquired. This result is consistent with the outcome of a 2-year programme studying the effects of aerobics and weight training on BMD of young US women (aged 20–35 years), which also showed a significant gain in BMD (1·3 % – 5·6 % in the exercise group) (Friedlander *et al*. 1995). No follow-up study after exercise intervention has been reported. Cross-sectional studies have shown positive associations between previous physical activity and bone mineral status in young women (Teegarden *et al*. 1996), and between lifetime physical activity levels and current

bone mass in pre- or post-menopausal women (Halioua & Anderson, 1989; Ulrich *et al*. 1996). A prospective cohort study commencing in 1980 with endpoint measurement of BMD after 11 years follow-up (baseline age 9–18 years) in Finnish adolescents and young adults showed that the subjects with the highest exercise levels had significantly higher lumbar spine and femoral neck BMD than subjects with the lowest exercise level, and suggested that regular exercise is important in achieving peak bone mass (Välimäki *et al*. 1994). Physical activity was estimated at study entry and after 6 and 11 years in the study. From the above findings we can see that physical activity may contribute to a higher peak bone mass if the level is maintained.

Calcium supplementation

Ca, as the most important bone mineral and subject to insufficient dietary intake, has been extensively trialled as a supplement to improve bone mineral status in children and adolescents.

Reliable information about the Ca–bone relationship has been available from several published Ca supplementation studies of children and adolescents (Table 2) (Matkovic *et al*. 1990; Johnston *et al*. 1992; Lloyd *et al*. 1993, 1996*a*; Andon *et al*. 1994; Lee *et al*. 1994*b*,1995*a*; Bonjour *et al*. 1997; Nowson *et al*. 1997; Dibba *et al*. 2000) Out of the nine trials, eight, including both studies carried out in China, showed that an increased Ca intake was associated with higher bone mineral increases (compared with unsupplemented controls) of approximately 1–5 % depending on skeletal site.

Some of these intervention studies found that Ca intake had a stronger impact on BMD at the hip and radius, where cortical bone is predominant (Bonjour *et al*. 1997), whereas some studies showed the effect was more pronounced on spine BMD, where more trabecular bone is present (Lloyd *et al*. 1996*a*; Nowson *et al*. 1997). Effects of Ca supplementation appeared to be more marked in children on low Ca intakes according to Bonjour *et al*. (1997) who, in a 2–3-year intervention, compared the effects of supplementation with foods enriched with Ca from milk extract (unspecified), in Swiss adolescent low- and high-Ca consumers; and, in addition, according to Lee *et al*. (1994*b*, 1995*a*), who studied Chinese children accustomed to a low-Ca diet and to Dibba *et al*. (2000) who supplemented Gambian children also accustomed to a low-Ca intake.

Nowson *et al*. (1997) found a significant within-pair difference in BMD of spine and hip after 6 months Ca supplementation even though 74 % of their subjects had already achieved menarche, and they were unable to detect a significant effect of Ca on bone density in their premenarcheal twins (eleven pairs). However, in the study of forty-five pairs of identical twins by Johnston *et al*. (1992), adding 718 mg Ca/d to the diet of pre-pubertal children significantly increased bone density at five of six sites (average increase by 2·9 %) compared with controls at the end of the 3-year trial while among twenty-three peri-pubertal or post-pubertal pairs, the same amounts of the supplement demonstrated no benefit. Since other supplementation trials carried out in children aged 7–12 years with different pubertal status have all shown a positive effect, the optimal timing for Ca supplementation still needs further study, preferably with children accustomed to low- and high-Ca intakes.

The mechanism by which Ca supplementation enhances bone mass is not very clear. A reduced rate of bone turnover in supplemented subjects as shown by a reduced serum level of osteocalcin (bone formation marker) by Dibba *et al*. (2000) and Johnston *et al*. (1992) may be one of the reasons, because a reduced rate of bone turnover was related to increased bone mass in studies of black children and exercise subjects (Dalsky *et al*. 1988; Slemenda *et al*. 1997). However, as noted later, no effect on osteocalcin was shown in a UK supplementation trial of milk (as opposed to Ca) (Cadogan *et al*. 1997). Nowson *et al*. (1997) reported that the effect of

Ca supplementation on BMD was evident within the first 6 months and there was no further increment effect. This result supports the hypothesis that Ca supplementation increases bone density by suppressing bone remodelling and decreasing the remodelling space.

Whether the gain observed from the supplementation trials mentioned earlier persists to contribute to the peak bone mass is uncertain if all of the data from the few (five in number) long-term studies with follow up after Ca-supplement withdrawal are considered. Four of them (two in Chinese children) showed that the benefits of Ca supplementation on bone mass accumulation disappeared 12–36 months after supplements were withdrawn (Lloyd *et al*. 1996*b*; Lee *et al*. 1996, 1997; Slemenda *et al*. 1997). The fifth study indicated, according to our stricter interpretation of the data, that the difference in mean BMD gain was still detectable 12 months after termination of the Ca supplementation ($P<0{\cdot}05$), only for femur BMC which was still significantly higher ($P<0{\cdot}02$) in the spontaneously low-Ca consumers (Bonjour *et al*. 1997). These results overall are not very encouraging of short-term or even longer-term Ca supplementation given the results of Lloyd *et al*. (1996*b*), who ultimately divided their original cohort of about 100 girls into four groups, one of which was supplemented with 500 mg Ca/d for 4 years. Since most of these studies were conducted in pre-pubertal children, much longer supplementation trials with follow-up study at later ages (e.g. post-puberty) are needed to determine the long-term effects of Ca-supplementation on peak bone mass. Such studies would clearly be difficult to carry out given the sample numbers, compliance and long-term follow-up period required.

Lee *et al*. (1997) found that increased bone mineral acquisition during Ca supplementation of Chinese children appeared to be balanced by a reduced acquisition rate during the follow-up phase. These studies are of particular interest since Chinese children are accustomed to low-Ca intakes. Lee *et al*. (1994*a*,*b*, 1995*a*,*b*) also studied true fractional Ca absorption using stable isotopes in Chinese children in Hong Kong and southern China at baseline and after supplementation with Ca. There was no difference between Chinese and Hong Kong children. However, when data were considered on the basis of Ca intake, those with a lower intake (<500 mg/d) had a significantly higher true fractional Ca absorption than those on a higher intake (>500 mg/d). After 6 months Ca supplementation, true fractional Ca absorption of the study group was significantly lower than that of unsupplemented controls ($P=0{\cdot}001$). These results further suggest that supplementation may cause changes in the bone remodelling process.

The loss of the effects of Ca supplementation after the cessation of the intervention, while disappointing, is in accordance with the understanding of Ca homoeostasis in which bone acts as a reservoir of Ca to be drawn upon to maintain the serum ionised Ca when intake is low (Parfitt, 1987). The potential effects were shown by Bonofiglio *et al*. (2000) working with low- and high-Ca intake adolescent girls. Those with inadequate Ca intakes (387 mg/d) had normal radial bone density and levels of bone biomarkers, but elevated levels of parathyroid hormone, leading the authors to suggest that adequate Ca intakes would be needed to achieve peak bone mass as continuous hypersecretion of parathyroid hormone could be deleterious in the long term. It is not known if the overriding influence of serum ionised Ca would act to negate bone benefits which may be found after long-term supplementation. The question therefore arises whether optimum bone health in females can only be fostered by lifetime high-Ca intakes (at the upper end of current recommendations) possibly beyond those achievable via food alone.

Vitamin D supplementation

The role of vitamin D in mineralization of bone and prevention of rickets in infants and young children is well accepted worldwide, with the need for vitamin D supplementation and regular

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exposure to u.v. light recognised within the public health policies of most countries where sunshine is not abundant. Nowadays, the possibility of a role for vitamin D deficiency in osteoporosis in the elderly is becoming accepted with recommendations to supplement this group with Ca and vitamin D together (Reid, 1998). However, vitamin D status is often assumed to be replete in school-age children and young adults and there appear to have been few studies of the specific role of vitamin D supplementation in peak bone mass development in adolescence.

In the dairy-products intervention study conducted by Chan *et al*. (1995) over 1 year in healthy white US girls aged 11 years, the increase in dietary vitamin D of 4 μg/d, was positively associated with the observed gain in total body bone mineral (r 0·32, P<0·01) and lumbar spine BMD (r 0·41, P<0·01). (Other nutrients such as increased Ca, P and protein intakes in this study also produced favourable bone outcomes, but the relative efficacy of the different nutrients could not be tested.)

Zamora *et al*. (1999) compared the BMD at six skeletal sites of 8-year-old Swiss girls who had been breast-fed as infants, according to whether or not they had been supplemented with vitamin D in the first year of life. The supplemented and unsupplemented groups of girls did not differ in terms of age, Ca intake, demographic or anthropometric characteristics. The supplemented girls (n 91) had significantly higher BMC at the femoral neck and femoral trochanter than the unsupplemented girls (n 15) while areal BMD was significantly higher also at the radial metaphysis. A vitamin D-supplementation study by Docio *et al*. (1998) of pre-pubertal boys and girls showed some evidence of low vitamin D status in winter in normal Spanish children, while supplemented children improved their serum levels of $1,25(OH)_2D$ and decreased their parathyroid hormone levels (with outcomes depending on baseline 25(OH)D levels). Bone measurements were not made in this study and indeed the authors noted that: 'Lacking a long prospective study comparing vitamin D levels during the growing years and the peak bone mass attained after adolescence, the relationship between such subclinical vitamin D deficiency and peak bone mass remains speculative'. However, a study of Finnish children by Lehtonen-Veromaa *et al*. (1999) in the period February 1997 to March 1998 showed no effect of 3-months winter supplementation (October 1997–January 1998) of vitamin D (10 μg/d) on serum levels of 25(OH)D at follow-up in March 1998. Further, it should be noted, that in a study of young adult females in Germany, seasonal variations in vitamin D status and Ca absorption did not influence bone turnover (Zitterman *et al*. 1998) and the authors did not support vitamin D supplementation in such high-Ca consumers (>1200 mg/d).

Since Ca utilization depends on vitamin D supplies, intervention trials directed to measuring bone variables would also need to measure baseline and post-intervention Ca and vitamin D intakes, u.v. exposure, skin 7-dehydrocholesterol conversion and biochemical measures of Ca and vitamin D status to clarify the roles of the various factors.

There is always need for caution in vitamin D supplementation practice, certainly in terms of massive supplementation. Long-term vitamin D supplementation even at recommended levels is considered to be a possible hazard in terms of cardiovascular health (Fraser, 1995). However, it is interesting to note that Boucher (1998) has described a potential mechanism by which inadequate vitamin D status may contribute to insulin resistance and in a large European multicentre case–control study, vitamin D supplementation appeared to be protective against the emergence of type 1 diabetes (Anonymous, 1999).

Milk and dairy products supplementation

The fact that milk is a cheap, highly bioavailable and low-risk source of Ca renders the study of milk intake and bone mass of great interest.

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Table 3. Effects of milk supplementation on bone measures in children and adolescents

Reference	Country	Sex	*n*	Age (years)	Ca (mg/d) Diet	Ca (mg/d) Supplement	Duration (months)	Effects on bone: Forearm	Femur	Lumbar spine	Total body
Cadogan *et al.* (1997)	UK	F	82	12	745	316 ml milk (+386 mg Ca)	18	0	+	0	+
Chan *et al.* (1995)	USA	F	48	11	728	Milk/ cheese/ yogurt (+709 mg Ca)	12	0	–	+	+
Lau *et al.* (1992)	Hong Kong	M,F	114	14	340	250 ml milk (+176 mg Ca)*	12	0	–	–	–
Matkovic *et al.* (1990)	USA	F	10	14	750	900 ml milk (+756 mg Ca)	24	0	–	0	–

F, female; M, male; +, positive effect; –, not measured; 0, no effect.
* Milk supplement/school day; calcium intake expressed as mean intake/d over 12 months.

Four milk supplementation trials have been reported (Table 3) (Matkovic *et al*. 1990; Lau *et al*. 1992; Chan *et al*. 1995; Cadogan *et al*. 1997). The two most recent trials showed that increased milk or dairy products consumption enhanced bone mineral acquisition in adolescent girls significantly and more strikingly (1–9 %) than trials using Ca alone (1–5 %), while the earlier trials showed no effect. Some have concluded that supplementing with milk rather than Ca alone is therefore preferable (Kerstetter, 1995) but this conclusion is difficult to sustain from such a small number of milk trials especially given the variation in subject numbers, amount and duration of milk supplementation.

A multifactorial nutrition intervention such as milk supplementation is inevitably complex to interpret. For example, the intervention reported by Cadogan *et al*. (1997) showed a link with increased insulin-like growth factor-I. It has also been suggested that findings from this study may in part be explained by the additional cereal consumption by the milk group (New *et al*. 1998). Milk is a food containing many nutrients, other than protein, Ca and vitamin D, which favour bone growth. For example, lactose can increase the absorption of Ca. In the study conducted by Chan *et al*. (1995), intakes of dietary P, vitamin D and protein, which are present in significant amounts in dairy products, were also positively associated with the increases in lumbar spine BMD and total body bone mineral. The only study to have compared the effects of milk supplementation and calcium carbonate supplementation was too small (twelve or less subjects per group) to produce meaningful results (Matkovic *et al*. 1990).

As a major element of hydroxyapatite, P is also an important constituent of bone. Increased P intake can decrease intestinal Ca absorption and decrease renal excretion of Ca, but these effects may offset each other, and the overall Ca balance may not be affected (Arnaud & Sanchez, 1996). In the normal diet, Ca intake appears to be a more important factor in bone mineralization than phosphate intake (Wical & Brussee, 1979; Spencer *et al*. 1986). Dairy products are also a good source of vitamin D necessary for Ca intestinal absorption and bone formation. Protein may affect bone mass accumulation through insulin-like growth factor-I. Studies in both human subjects and animal models showed that insulin-like growth factor-I production was associated with dietary protein and energy intake and, of the two, protein intake appeared to play a more important role (Ross & Buchanan, 1990). Insulin-like growth factor-I promotes skeletal growth by stimulating osteoblast proliferation and differentiation and matrix formation, including the synthesis of type I collagen and other protein components (Price *et al*. 1994). High protein intake has a negative effect on Ca balance by increasing urinary Ca excretion.

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However, if the phosphate intake increases with the protein intake, the effect of a high protein intake on Ca metabolism can be offset, because P can decrease urinary Ca excretion (Hegsted *et al*. 1981; Schuette & Linkswiler, 1982). Whether high protein intake will have harmful results on Ca balance also depends on dietary Ca adequacy. At high Ca intake, the protein-induced Ca loss can be offset by improved absorption efficiency, whereas at low Ca intake, this kind of adjustment is not sufficient to offset the Ca loss induced by protein. Therefore, the dietary Ca:protein ratio may be important in achieving a positive Ca balance, and the suggested ratio is at least 16:1 (mg:g) according to the current recommended dietary allowance (Heaney, 1993*a*,*b*, 1998). The high protein content in dairy products should not affect Ca retention because of their abundance of Ca relative to protein (36:1) (Heaney, 1993*b*).

There appear to be no long-term studies of milk supplementation, nor follow-up studies after cessation of a milk supplement. Such studies would probably be difficult to carry out but are clearly needed if proper recommendations for milk consumption throughout childhood and adolescence are to be made. From empirical observation of the effects of cessation of a Ca supplement (see earlier), one can hypothesise that the benefits of milk supplementation would also be lost after cessation.

While many support increased milk intakes in children and adolescents as an effective way to promote bone health, there are still concerns related to this approach. One such concern is lactose intolerance, the inability to digest the milk sugar lactose because of a deficiency or defect in intestinal lactase. The usual symptoms of lactose intolerance are bloating, abdominal pain, diarrhoea, and flatus. It is generally considered that the rate of lactose intolerance is high in Asian populations. However, according to the cross-sectional study carried out in 1300 Chinese adolescent girls by Du (1998), only 13 % reported symptoms indicative of lactose intolerance. Lactose intolerance has been reported to cause few problems if milk intake is limited to 240 ml/d, especially when the milk is taken with meals (Scrimshaw & Murray, 1988; Suarez *et al*. 1995).

Another concern is that dairy products may lead to a high fat intake due to its 3·5–4·0 % butterfat content. However, in the study carried out by Chan *et al*. (1995) there was no difference in fat consumption (expressed either as total fat (g), saturated fat (g), or % total energy as fat) between the supplementation and control group. In milk supplementation studies (250–900 ml milk/d) there was no difference in body composition or weight gain between the intervention and control groups at the end of the trial. One possible explanation for these findings is that the addition of milk or other dairy products to children's diet may cause a conscious or subconscious change in the overall diet, so that fat intake remains relatively constant. The effect of dietary Ca on Fe absorption should also be taken into account when considering milk supplementation, because Fe deficiency is also a major nutritional problem in children, adolescents, menstruating and pregnant women, and Ca has been reported to have adverse effects on Fe absorption. Several studies have shown Ca from both supplements and dairy products (milk, cheese) inhibited non-haem-Fe absorption when added to meals (Dawson-Hughes *et al*. 1986; Deehr *et al*. 1990; Cook *et al*. 1991; Hallberg *et al*. 1992). However, not all studies have found that Ca intake inhibits Fe absorption. In a study conducted in fourteen volunteers (males and females) aged from 19 to 37 years, Ca intake had no significant influence on non-haem-Fe absorption from a complete diet (Reddy & Cook, 1997). A further study in Sweden using chemical balance techniques found no decrease in apparent Fe absorption in ileostomy subjects aged 29–63 years during a high-Ca (milk) diet (Tidehag *et al*. 1995). It is suggested that Fe bioavailability could be enhanced by separating Ca and Fe intakes, i.e. ingest most of the dietary Ca at meals containing the least dietary Fe (Gleerup *et al*. 1995).

On-going studies in our department

Most of the intervention trials except the two carried out in 7-year-old Chinese children by Lee *et al*. (1994*b*,1995*a*) were conducted in Western countries in well-nourished children and adolescents, in populations accustomed to consuming milk and its products. The data of Du *et al*. (1997*a*) showed that adolescent girls in northern China had poor Ca intake (350 mg/d) and high prevalence rate of clinical and subclinical Ca and vitamin D deficiency in winter (6 % and 51 % respectively), and also showed that milk intake (although low) was the major dietary determinant of bone mineral status, independent of Ca and vitamin D intakes (Du *et al*. 1999). These findings indicated that nutritional intervention was warranted to promote the bone health of Chinese adolescent girls.

We have commenced a randomized double-blind milk supplementation trial over 2 years in Chinese pre-adolescent schoolgirls. Schools have been randomized into three groups: control schools; milk-supplementation schools (330 ml Ca-fortified ultra-heat-treated milk per school day); milk- and vitamin D-supplementation schools (330 ml Ca- and vitamin D-fortified ultra-heat-treated milk per school day). All girls aged 10–11 years in these schools are included in the trial, subject to parental consent being given. The trial has received ethical approval from the University of Sydney, Australia and the Institute of Nutrition and Food Hygiene in Beijing. The milk has been specially formulated (Murray Goulburn Cooperative Limited, Australia) to deliver 8 μg vitamin D and 560 mg Ca/d (the equivalent of the Ca in 500 ml regular milk) to each subject, within a volume of 330 ml. This is in order to keep the supplementation milk within the amount readily consumed on each occasion and to avoid any problems of lactose intolerance associated with milk overload. In a pretrial of the milk forty-seven boys and forty girls aged 10 years (in a different school from those in the supplementation trials) were asked to consume a 330 ml package (15·2 g lactose) and record their reactions to it. Fewer than 5 % of these children were classified as lactose intolerant (criteria were having at least three symptoms after drinking the milk; or having at least two symptoms after drinking the milk together with a history of sickness after drinking milk). Among the 500 children originally targeted to receive the supplementation there have been very few drop-outs related to the acceptability of the daily package of milk (Greenfield *et al*. 1999).

Preliminary analyses of the baseline data recorded prior to trial commencement have shown that serum osteocalcin and insulin-like growth factor-I were significantly correlated with total body and radial BMD of the subjects, and that all bone biomarkers studied were at substantially higher levels than those of Chinese adults (Zhu *et al*. 2000). Results after only 12 months supplementation show that the milk-supplementation group had a greater percentage increase in distal forearm BMD (7·73 *v*. 5·48, P=0·001), and the milk- and vitamin D-supplementation group had a greater percentage increase in total body BMD (9·75 *v*. 7·99, P=0·001) in comparison with the control group (Zhu *et al*. 2000).

The study incorporates the collection of a battery of socio-demographic, biochemical and genetic data. Thus by the end of the trial, we hope to shed light on the effects of milk and vitamin D supplementation on bone mass accumulation in adolescent girls accustomed to a low-Ca diet and with poor vitamin D status; the relative importance of Ca and vitamin D in bone mass accumulation; and the ways in which Ca and vitamin D supplementation affect bone turnover; and, specifically, the response of different genotypes to milk supplementation. More importantly, we hope to be able to follow-up supplemented and unsupplemented girls for 2 years after the milk supplementation trial ceases with the objective of ascertaining whether the effects of short-term supplementation are sustainable. The information produced will inform the health, agricultural and educational authorities in China, where there is a strong groundswell of interest in implementing school milk programmes nationally (Anonymous, 2000). Is there a need for

such programmes at primary school level only, or should they endure throughout schooling? This is particularly important given the inadequacy of current levels of milk production in China to meet such needs, the shortfall on 1997 data being calculated as 1·8 million tonnes annually (Greenfield *et al*. 1999).

Conclusion

In conclusion, adolescence is the crucial time for peak bone mass attainment. Genetic components are substantial determinants of bone mass acquisition during this period, and but prepuberty appears to be the most opportune time for modifying bone mass by environmental factors. Continuing work is needed to explore the mechanism of bone development perhaps with more focus on bone geometry and structure than in the past (Schönau *et al*. 1996; Seeman, 1997) to deal with the many unanswered questions about how genotype and environment interact particularly in countries with marginal child nutrition. In Asia, where osteoporosis has been predicted to be a health disaster waiting to happen, more information is needed about bone metabolism especially in view of the assertion that the adult Chinese absorb Ca more efficiently (Kung *et al*. 1998) and that Ca absorption from low-oxalate Chinese vegetables is as high as that from milk (Weaver *et al*. 1997).

In general, short-term intervention trials, including those in low-Ca consumers, have shown that exercise, Ca and milk supplementation have beneficial effects on bone mass accumulation during childhood and adolescence, and, if this effect could be maintained, will favour the attainment of a higher peak bone mass. Far more study is needed to clarify the mechanisms and long-term effects of such interventions; however, it can provisionally be concluded that encouraging physical activity, Ca and milk intake in children and adolescents will reduce their osteoporosis risk in the future to some extent. Since physical activity and milk are low risk interventions, in tune with the general tenor of nutritional recommendations generally, there is no reason why they should not commence now. The school environment offers supervision and integration of such interventions into the health education syllabus. Multi-strategy programmes in schools, for example, involving school milk, appropriate physical activity and judicious sunlight exposure, may be an effective route to intervention (H Greenfield, X Du, K Zhu and DR Fraser, unpublished results) but, according to current theories of health promotion (Baum, 1998), will require a broad framework of school community participation if they are to be implemented successfully.

Acknowledgement

Zhu Kun and Du Xueqin and costs of their project work in China are supported by the Australian Dairy R&D Corporation.

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