Proceedings of the Nutrition Society (2001), **60**, 45–52 DOI: 10.1079/PNS200072

The Summer Meeting of the Nutrition Society was held at University College, Cork, Republic of Ireland on 27–30 June 2000

Micronutrient Group and Reproduction and Development Group Symposium on 'The relative contribution of diet and genotype to development'

The relative contribution of diet and genotype to bone development

Ann Prentice
MRC Human Nutrition Research, Downham's Lane, Milton Road, Cambridge CB4 1XJ, UK

The present review addresses the relative contribution of diet and genotype to variability in human bone growth and mineralisation in the context of the aetiology of osteoporosis. Heritability studies indicate that 60–70 % of the variability in bone mineral mass or bone mineral density (BMD) can be accounted for by genetic variation. Cross-trait analyses suggest that a proportion of this variation reflects genetic influences on bone and body size, such as height and lean body mass. Candidate-gene studies have demonstrated associations between several genetic polymorphisms and bone mineral mass but, as yet, genotype determinations have proved unhelpful in identifying individuals at increased risk of osteoporosis. Variations in diet and other environmental factors contribute 30–40 % to total phenotypic variance in bone mineral mass or BMD. Correlations between intakes of individual nutrients and BMD have been reported, but these relationships are subject to confounding due to size. However, no specific dietary factor has been identified from prospective and twin studies as making a significant contribution to environmental variability in BMD or bone loss. This finding may reflect the difficulties in quantifying environmental exposures, both current and over a lifetime. In addition, the influence of diet on bone health may depend on the genotype of the individual. Optimisation of nutrition and lifestyle remains an attractive strategy for the reduction of fracture risk, but more research is required to fully define optimal dietary requirements.

Osteoporosis: Bone mineral mass: Bone mineral density: Candidate genes

Bone is a specialised connective tissue that, together with cartilage, forms the skeletal system. The skeleton functions as a mechanical support for the body, protecting the vital inner organs and facilitating muscle action and locomotion, and acts as a metabolic reservoir of ions, especially Ca and phosphate, for the essential preservation of extracellular homeostasis. The morphogenesis, growth, development and subsequent health of bone are influenced by a myriad of genetic, cellular, hormonal and environmental factors. The present review focuses on the relative contribution of diet and genotype to variability in human bone growth and mineralisation in otherwise healthy individuals within the context of the aetiology of osteoporosis.

Human bone growth and development

A discussion of bone development requires knowledge of human bone biology and terminology. The following description aims to provide a brief overview of the main concepts. Fuller explanations can be found in good text-books; sources used to prepare the present summary were: Nilsson *et al*. 1994; Prentice & Bates, 1994; Price *et al*. 1994; Marcus *et al*. 1996; American Society for Bone and Mineral Research, 1999.

Bone consists of collagen fibres, of which 90 % are type 1, a mineral phase of crystals of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) and other ions, and a ground substance formed by glycoproteins and proteoglycans. Three cell types produce and maintain bone. Osteoblasts (bone-forming

Abbreviations: BMD, bone mineral density; DZ, dizygotic; IGF, insulin-like growth factor; *INS* VNTR, insulin gene variable number of tandem repeats locus; MZ, monozygotic; VDR, vitamin D receptor.

Corresponding author: Dr Ann Prentice, fax +44 1223 437515, email ann.prentice@mrc-hnr.cam.ac.uk

cells) work at bone surfaces where they secrete collagen and ground substance, influence the crystallisation of hydroxyapatite and modulate the activity of bone-resorbing cells. Osteocytes are osteoblasts that have become embedded within the calcified regions of bone. These cells continue to be metabolically active and are involved in the sensing and communication of information about the internal bone environment. Osteoclasts (bone-resorbing cells) are responsible for the resorption (destruction) of bone that is a necessary first step in the repair of bone surfaces and the remodelling of bone. This process results in a controlled coupled system of bone resorption followed by bone formation, which models growing bone and remodels existing tissue.

The skeleton contains two bone types: flat bones of the axial skeleton (e.g. pelvis, vertebrae) and the long bones of the appendicular skeleton (e.g. femur, radius). Flat bones develop and grow by a process of intramembranous ossification. Mesenchymal cells proliferate and differentiate into pre-osteoblasts and osteoblasts within a highly-vascularised region of embryonic connective tissue. These cells produce woven bone in which collagen fibres are randomly orientated and calcification occurs in irregular patches. Woven bone is eventually remodelled to produce lamellar bone, in which collagen fibres are orientated in specific directions and packed in layers where the orientations alternate. Lamellar bone is organised into osteons, cylindrical structures surrounding Haversian canals which contain blood vessels and nerves.

Long bones develop in length by a process of endochondral ossification within a layer of hyaline cartilage (epiphyseal growth plate) located towards the ends of the long bones. During this process, cartilage in the portion of the growth plate adjacent to the bone shaft matures, the tissue becomes calcified, and is subsequently resorbed and replaced by bone. Concomitantly, new chondrocytes proliferate in the portion of the growth plate adjacent to the ends of the long bones. By this means, growth in bone length and overall statural height is achieved. At puberty, the growth plates becomes fully calcified (fused) and linear growth stops.

Long bones develop in width by intramembranous ossification involving bone formation (apposition) on the periosteal (outer) surface. Unlike endochondral ossification, periosteal apposition does not stop at puberty and the width of bones (bone envelope) increases slowly throughout life. Apposition can also occur at the endosteal (inner) bone surface, depending on the stage of life, while bone resorption occurs at other times (Garn, 1970). As a result, the volume of cortical bone within the bone envelope expands and contracts depending on the stage of life. It has been proposed that the expansion by endosteal apposition at puberty in girls acts as a reservoir for the minerals required for reproduction, and that it is this reservoir which is lost at the menopause (Schiessl *et al*. 1998).

Within each bone, bone tissue is organised into two different structures, the proportions of which vary between regions of the skeleton. Cortical (compact) bone is a thick and dense layer of calcified tissue that forms the outer surfaces of most bones and the shafts of the long bones. Trabecular (cancellous) bone has a spongy appearance and consists of a lattice of thin calcified trabeculae. A high proportion of trabecular bone is found at the ends of long bones and within flat bones and the vertebrae. The spaces between the trabeculae are filled with haematopoietic bone marrow, which produces blood cells and precursors of osteoblasts and osteoclasts, and contains modulators of cell proliferation, differentiation and longevity. Bone marrow is also located in the medullary cavity of long bones. Cortical and trabecular bone are constructed from the same cell types and matrix elements but they differ structurally, both in their spatial arrangement and in tissue calcification. In cortical bone 80–90 % of the volume is calcified, whereas the percentage is only 15–25 in trabecular bone, the remainder being occupied by blood vessels, connective tissue and bone marrow. The main interface between bone and soft tissues occurs at the endosteal (inner) surfaces of the skeleton where the proportion of trabecular bone is greatest. As a result, the main function of trabecular bone is metabolic, whereas the role of cortical bone is predominantly structural.

Embryonic development of the skeleton begins with the differentiation of cells into chondrocytes. The notochord emerges by the second week of gestation and limb buds appear early in the second month. Intramembranous and endochondral ossification takes place, and epiphyseal growth plates emerge. A mineralised skeleton becomes evident by the second half of gestation, and mineral accretion is at its greatest in the third trimester. At birth the skeleton contains about 25 g Ca (Widdowson & Dickerson, 1964).

Bone mass increases during childhood and adolescence. Accretion rates are highest in infancy and during the pubertal growth spurt, with lower rates in childhood. The epiphyses fuse at the end of puberty, but bone mass continues to increase for several years after linear growth stops, reaching a peak during young adulthood. The age at which peak bone mass is achieved varies between different regions of the body and different populations. Studies in young men and women have reported continuing increases in bone mineral mass at the whole-body, spine and appendicular skeleton during their early twenties, while decreases are seen at the femoral neck from the mid-teenage years (Bonjour *et al*. 1991; Parsons *et al*. 1996). After peak bone mass there is a slow decline in bone mineral mass which is accelerated in women in the years following the menopause. This loss occurs by resorption at the endosteal surfaces; periosteal apposition continues (see earlier). The rates of post-menopausal bone mineral loss average 1–2 %/year in cortical bone and 2–3 %/year in trabecular bone. These age-related changes in bone mineral mass match those occurring in total skeletal mass as the mineral:protein matrix remains broadly similar.

The growth, development and subsequent maturational changes of bone are under endocrine control. The key systems are the growth hormone–insulin-like growth factor (IGF)-1 axis, the gonadal axis and pituitary–thyroid axis, although other hormones such as insulin are also involved. In addition, the hormones involved in Ca homeostasis regulate the inflow and efflux of Ca and phosphate from bone. During fetal life growth is independent of growth hormone and hormones such as IGF-1, IGF-2 and insulin

are important modulators. During childhood the central regulators are growth hormone and IGF-1. These hormones induce linear growth by the proliferation of cells of the epiphyseal growth plate without promoting maturation. Thyroid hormones, acting via nuclear receptors, are important for the normal proliferation and maturation of the growth plate. During puberty sex hormones promote proliferation with maturation, resulting in a growth spurt that ends with fusion of the growth plate. Recent evidence indicates that it is the oestrogenic hormones that play the key role in limiting linear growth, as epiphyseal fusion is delayed in male subjects who lack either aromatase, the enzyme that converts testosterone to oestrogen, or functional oestrogen receptors (American Society for Bone and Mineral Research, 1999). The decline in sex hormones in older individuals, especially that of oestrogen at the menopause, is associated with age-related bone loss.

Disorders of human bone growth and development

Normal bone growth and mineralisation are the result of a complex interplay of genetic, cellular, hormonal and environmental influences. Disturbances at any level can result in bone abnormalities and disease. Much information about normal bone development has been obtained from the study of inherited and acquired bone and endocrine disorders. Examples include osteogenesis imperfecta (brittle bone disease) caused by abnormalities in the structure and production of type 1 collagen, hypophosphatasic rickets, caused by subnormal activity of a specific isoform of alkaline phosphatase, and the excessive and impaired secretion of growth hormone producing gigantism and poor growth respectively. Physical activity and a supply of essential nutrients are also prerequisites for bone development. Paralysis of a limb compromises skeletal growth and promotes involution. Malnutrition impairs growth, and frank deficiencies of a wide range of nutrients can compromise skeletal health. Two well-known examples, among many, are that a lack of vitamin C, a cofactor for the enzyme lysyl oxidase that produces collagen cross-linking, results in scorbutic osteopenia, while a lack of vitamin D results in rickets (children) or osteomalacia (adults) due to aberrant production of bone protein matrix and defective mineralisation.

Osteoporosis is characterised by loss of bony tissue from the skeleton and deterioration of bone structure, and is associated with enhanced bone fragility and increased risk of fracture, most commonly at the wrist, vertebrae and hip. Primary osteoporosis is a feature of the ageing process, especially in women after the menopause, and older individuals are prone to fragility fractures. In the UK one in three women and one in twelve men over the age of 50 years can expect to experience an osteoporotic fracture during the remainder of their life. These fractures are accompanied by significant morbidity, reduced quality of life and, in the case of hip fractures, increased mortality risk, making osteoporosis an issue of major public health concern.

Increased susceptibility to fragility fractures in older individuals is associated with a range of risk factors which includes low bone mineral mass or BMD, short stature, low body weight or muscle mass and differences in bone proportions (Compston, 1993; Cummings *et al*. 1993; Bass *et al*. 1999). These traits are, to some extent, expressions of variability in bone development at different stages of life. Understanding the relative contribution of diet and genotype to the variability of these traits may provide valuable insights into the aetiology of osteoporosis. A significant genetic component is suggested by the fact that a family history of fractures is a recognised risk factor for osteoporosis, and that relatives of patients with osteoporosis have lower bone mineral mass or BMD than those of controls (Evans *et al*. 1988; Seeman *et al*. 1989, 1994; Soroko *et al*. 1994).

Heritability of a trait

Heritability of a trait is the proportion of the variability of that trait within a population that can be attributed to genetic variation as opposed to environmental factors and measurement error:

$$\text{heritability } (\%) = (V_g/V_p) \times 100,\ V_p = V_g+V_e+V_m,$$

where V_p is total phenotypic variance, V_g is genetic variance, V_e is environmental variance and V_m is variance due to measurement error (Seeman & Hopper, 1997). Since heritability is a proportion, the estimate is not fixed and can vary, for example between different populations and different ages. Such variations may reflect differences in the partitioning of the variance between the various components or differences in total phenotypic variance. If, for example, the genetic variance of a trait remains the same but environmental variance increases, total phenotypic variance becomes greater and the estimate of heritability goes down. In addition, estimates of heritability depend on the precision of the measurement, since measurement error contributes to the denominator. If the error with which the trait is quantified is high, the estimate of heritability is lower than it otherwise would be.

It is important to note that heritability provides an estimate of the possible causes of variation in a trait within a specified population, and does not describe the extent to which genetic factors determine the trait itself or the incidence of disease. An estimate of 70 % heritability for stature or fracture incidence, for example, indicates that 70 % of the variability is associated with genetic factors, but does not mean that 70 % of the final attained height or of the number of fracture cases is genetically determined (Hopper, 1993; Seeman & Hopper, 1997).

Estimates of heritability are generally obtained by correlation studies either of pairs of twins (the classic twin model) or of relatives within families. Monozygotic (MZ) twins share 100 % of their genes and, therefore, differences between them are due to environmental influences (including measurement error), whereas dizygotic (DZ) twins share only 50 % of their genes and differences between them indicate a combination of genetic and environmental influences. In the classic twin model the extent to which MZ twins are more alike for a given trait than DZ twins is taken as a measure of the genetic contribution to the variability of that trait. This measure is obtained by comparing the intraclass correlations for each

https://doi.org/10.1079/PNS200072 Published online by Cambridge University Press

zygosity (Arden & Spector, 1997). If the only reason why a trait is correlated between twins is genetic, then the correlation between MZ twins will be double that of DZ twins. A smaller difference indicates the involvement of environmental influences. The classic twin model makes three assumptions: (a) that the total phenotypic variances of MZ and DZ twins are equal; (b) that the variances due to shared environment in MZ and DZ twins are equivalent; (c) that any genetic variance is additive, i.e. there are no interactions between genes. Violations of these assumptions lead to over- or underestimates of heritability.

Familial resemblance studies compare the trait between and across generations within families (sibling *v.* sibling and parent *v.* child correlations). Estimates of heritability by intergenerational studies can be complicated by uncertainties over parentage, the possibility of assortative mating (parent *v.* parent correlations), the likelihood of a degree of shared environment within families, and by potential differences in environmental influences between birth cohorts and between age-groups which alter total phenotypic variance.

Heritability of variation in bone mineral mass or bone mineral density

Strong correlations in indices of bone mineral mass or BMD and rates of bone loss are found between MZ and DZ twins (Smith *et al*. 1973; Pocock *et al*. 1987; Pollitzer & Anderson, 1989; Arden *et al*. 1996; Flicker *et al*. 1997; Harris *et al*. 1998; Howard *et al*. 1998) and between first-order relatives (Lutz, 1986; Pollitzer & Anderson, 1989; Tylavsky *et al*. 1989; Kelly *et al*. 1993; Krall & Dawson-Hughes, 1993; Danielson *et al*. 1999; Magarey *et al*. 1999). Comparisons of intraclass correlations between zygosities in twin studies give heritability estimates for bone mineral mass or BMD of about 70–80 %. These estimates are higher than the 50–60 % obtained with family studies. It has been argued that twin studies give unrealistically high estimates, suggesting that the assumptions of the classic twin model are violated (Slemenda *et al*. 1991). Possible explanations include the high probability that MZ twins have a greater degree of shared environment than DZ twins, with a consequent over-attribution of similarities to genetic factors (Slemenda *et al*. 1991; Seeman & Hopper, 1997), and the likelihood of interactions between genes (Slemenda *et al*. 1991). On the other hand, it has been argued that the genetic contribution estimated from parent–offspring studies is diluted by intergenerational differences in total phenotypic variance (Smith *et al*. 1973; Seeman & Hopper, 1997) and by accumulated environmental influences making a larger contribution in older than younger individuals (Pollitzer & Anderson, 1989). Either way, these studies demonstrate that a sizeable percentage, probably about 60–70, of the variation in bone mineral mass or BMD is associated with differences in genetic make-up. It should be noted that several studies include adjustments for environmental factors such as smoking and use of oestrogen-replacement therapy. This procedure enables consideration of the genetic contribution in the absence of these environmental confounders, but inflates the heritability estimate by decreasing the total phenotypic variance of the population.

Most studies have been conducted by measuring areal BMD using absorptiometry. This index is a partial correction of bone mineral mass for scanned bone area and retains information about bone and body size (Prentice *et al*. 1994). Many studies have demonstrated correlations between BMD and other aspects of size, such as lean body mass, body weight, statural height and bone dimensions (Prentice *et al*. 1994; Henderson *et al*. 1995; Parsons *et al*. 1996; Arden & Spector, 1997; Molgaard *et al*. 1997; Magarey *et al*. 1999). Variability in these characteristics also has a sizeable genetic component. Heritability of stature is about 60–80 %, except in populations where adverse environmental factors have an impact on growth, such as those in rural Africa (Roberts *et al*. 1978). Heritability of lean body mass has been estimated at between 50 % and 80 % (Krall & Dawson-Hughes, 1993; Seeman *et al*. 1996; Arden & Spector, 1997). Cross-trait correlations in twin and family studies suggest that there are common genes regulating bone mineral mass or BMD and lean body mass (Young *et al*. 1995; Seeman *et al*. 1996; Seeman & Hopper 1997; Nguyen *et al*. 1998; Nordstrom & Lorentzon, 1999). Adjustment for these characteristics reduces the heritability estimates for BMD by 5–20 %, indicating that some of the genetic variability in bone mineral mass or BMD reflects genetically-determined variability in size. However, a degree of heritability remains, indicating that there is a genetic component to variability in BMD that is independent of size and muscle mass. In addition, there is evidence that familial resemblance is expressed in childhood before the pubertal growth spurt when, arguably, the contribution of environment to variation in growth is likely to increase (Ferrari *et al*. 1998).

Variability in genes involved in the regulation of bone formation and resorption may influence the phenotypic variance of BMD. Twin studies have demonstrated high heritability estimates for serum bone-specific alkaline phosphatase and osteocalcin, markers of osteoblast activity (Kelly *et al*. 1991; Harris *et al*. 1998). Cross-trait correlations, however, have found no evidence that variability of BMD is a direct consequence of the genetic regulation of bone turnover (Harris *et al*. 1998).

Contribution of genotype

There are two main strategies for identifying genes that influence a specific trait, the candidate-gene approach and genome screening (Nguyen *et al*. 2000). Many genes are involved in the regulation of bone mass, and the limitation of the candidate-gene approach is that individual genes are examined in isolation, whereas there is a high probability that variations in bone development and osteoporosis risk are likely to be polygenic. Nevertheless, to date, most studies exploring the genetic contribution to human variability in bone mineral mass or BMD and fracture risk have used the candidate-gene approach and have concentrated on a relatively small number of genes (Ralston, 1998). The gene that has received the most attention is the vitamin D receptor gene.

Vitamin D receptor gene

The vitamin D receptor (VDR) is a member of the superfamily of nuclear receptors that regulate gene expression in a ligand-dependent fashion. The receptor protein is located predominantly within the cell nucleus, even in its unbound state. The ligand for the VDR protein is the hormone 1α,25-dihydroxyvitamin D, produced by the hydroxylation of 25-hydroxyvitamin D in the kidney in response to parathyroid hormone. Binding of the hormone–VDR complex to DNA, after formation of a heterodimer with the retinoid X receptor, mediates the actions of 1α,25-dihydroxyvitamin D in the many cell systems in which it operates, not only those involved in the regulation of Ca and phosphate translocation, but also cells of the immune, neural, epithelial and endocrine systems (Haussler *et al*. 1998).

Four common single-nucleotide polymorphisms have been defined in the gene encoding the VDR protein, recognised by the restriction enzymes *Bsm*l, *Apa*l, *Taq*l and *Fok*l. The first three correspond to differences in non-coding regions of the gene, and these polymorphisms do not affect VDR protein structure but may alter gene expression. The *Fok*l polymorphism, on the other hand, represents a difference in the VDR translation initiation site where in the minority allele (f; presence of the restriction site) a cytosine → thymine transition creates an upstream initiation codon that produces a VDR protein that is three amino acids longer than that produced by the majority allele (F; absence of the restriction site; Pols *et al*. 1998; Ames *et al*. 1999). These two isoforms of the VDR protein may be functionally different (Arai *et al*. 1997).

A link between polymorphisms in the VDR gene and bone development was first reported by Morrison *et al*. (1992, 1994) who showed associations between the minority allele at the *Bsm*l restriction site (B; absence of the *Bsm*l restriction site) and lower BMD and higher serum osteocalcin concentrations, and hence a higher risk of osteoporosis. Corrections to errors in the original paper subsequently suggested a weaker effect (Morrison *et al*. 1997). The possibility that the B allele confers a greater risk of osteoporosis was also suggested by the fact that Chinese and African populations, who are less prone to hip fractures, have a lower frequency of the BB genotype (Beavan *et al*. 1996). Since that time a range of population studies have indicated either similar associations to those originally reported by Morrison *et al*. (1992, 1994), no associations or inverse associations (Cooper & Umbach, 1996; Ralston, 1998, 1999). The reasons for this situation are likely to be complex, and may include variability in gene–environment interactions (Pols *et al*. 1998). Overall, the current consensus is that there is a weak association between VDR polymorphisms and BMD (Pols *et al*. 1998; Ralston, 1998, 1999; Nguyen *et al*. 2000). This association may be mediated by effects on bone growth and size, as VDR genotype has been associated with differences in height during childhood, and in femur dimensions in older women (Heaney *et al*. 1997; Tao *et al*. 1998). However, as yet, there is no convincing evidence from case–control studies of an association between VDR genotype and fracture risk (Ralston, 1998; Ensrud *et al*. 1999).

Other candidate genes

Polymorphisms of several other candidate genes have been associated with bone mineral mass or BMD and/or fracture risk. These genes include those encoding for bone proteins, hormones and their receptors, cytokines, growth factors, enzymes and transporting factors involved in mineral and bone metabolism. Examples include polymorphisms in the genes for collagen-1-α-1, osteocalcin, the oestrogen receptor (both α and β), the calcitonin and parathyroid hormone receptors, interleukin-6, IGF-1, transforming growth factor-β and apolipoprotein E (Greenfield & Goldberg, 1997; Ralston, 1999). As yet these relationships are inconclusive, because of insufficient evidence and conflicting results between populations.

Recent evidence suggests that polymorphisms at the insulin gene variable number of tandem repeats locus (*INS* VNTR) influence fetal bone growth (Dunger *et al*. 1998; Ong *et al*. 1999) and consequently may affect later bone development. There are two main allele sizes for the *INS* VNTR: class I alleles contain twenty-six to sixty-three repeat units; class III alleles contain 141–209 repeat units. These polymorphisms have been shown to influence the transcription of insulin and IGF-2 (Ong *et al*. 1999). Class III alleles are associated with protection against insulin-dependent diabetes mellitus and with increased susceptibility to insulin-resistant states such as polycystic ovary syndrome and non-insulin-dependent diabetes mellitus.

Genetically-determined fetal insulin resistance could provide an explanation for the link between fetal growth and insulin resistance in later life (Hattersley & Tooke, 1999). In a study involving 758 children from the Avon Longitudinal Study of Pregnancy and Childhood cohort in Bristol, UK, class III homozygotes had greater head circumference, greater length and greater weight at birth (Dunger *et al*. 1998). The effect was stronger and more significant in those children whose postnatal growth pattern did not exhibit 'catch-up' or 'catch-down', as indicated by a change of ±0·67 SD, and in whom, therefore, growth was less likely to have been influenced by the maternal uterine environment. Since size at birth and in early childhood influences adult bone mineral mass and, hence, fracture risk (Cooper *et al*. 1995, 1997) it is possible that *INS* VNTR polymorphisms may have a role in determining bone health in later life.

Contribution of diet and other environmental factors

Heritability studies indicate that 30–40 % of the variation in bone mineral mass or BMD and in fracture risk is not accounted for by genetic factors and must be contributed by environmental factors and measurement error. A large number of environmental factors may be involved, including physical activity patterns, smoking, alcohol consumption, dietary intakes and diet composition (Department of Health, 1998). It has proved difficult to detect environmental influences on variations in bone health. This situation may reflect the difficulties in adequately quantifying environmental exposures, both current and lifelong, and in detecting environmental effects against a background of considerable trait variance caused by genetic factors. However, this outcome should not be

https://doi.org/10.1079/PNS200072 Published online by Cambridge University Press

interpreted as indicating that diet and environmental factors are not important in determining variation in susceptibility to osteoporosis; their effects may be small, yet prove to be important if the exposure is common (Seeman & Hopper, 1997).

Interest in the role of diet in determining variation in osteoporosis risk has centred largely on Ca (Prentice, 1997). Many epidemiological studies have demonstrated a weak association between BMD and Ca intake, but the variation in Ca intake only contributes about 1 % to the population variance. It is likely that this relationship largely reflects the confounding influence of size rather than a specific effect of Ca nutrition (Prentice *et al*. 1994; Prentice, 1997). To date, although adults with the lowest Ca intake within a population tend to have the lowest BMD and to be at the highest risk of osteoporosis (Cumming & Nevitt, 1997), twin studies have been unable to demonstrate a contribution of Ca intake to phenotypic variance in BMD (Young *et al*. 1995), and population-based prospective studies have failed to show an effect of customary Ca intake on variability in adult bone loss (Hannan *et al*. 2000). Increases in BMD have been observed in Ca supplementation studies, especially of young individuals and the elderly (Prentice, 1997). However, with few exceptions, the magnitude of the response to supplementation has not been shown to depend on customary Ca intake, so that similar responses are observed in those with the highest and lowest Ca intakes in the sample. This outcome makes it difficult to quantify the contribution of variation in Ca intake to variability in BMD and fracture risk, or to define dietary Ca requirements on the basis of optimal bone health (Department of Health, 1998).

It is possible that the extent to which dietary differences contribute to variability may be influenced by genetic variation. Ca–gene interactions have been reported for polymorphisms in the VDR gene. For example, the elevation in Ca absorption induced by Ca restriction was blunted in individuals with the BB genotype (Dawson-Hughes *et al*. 1995), and the effect of Ca supplementation in reducing bone loss at the femoral neck in post-menopausal women was only evident in women with the BB genotype who had a low customary Ca intake (Krall *et al*. 1995). Other nutrient–gene interactions have been observed. For example, the transport of vitamin K, an essential cofactor in the γ-carboxylation of osteocalcin, is influenced by the apolipoprotein E genotype (Saupe *et al*. 1993). However, such effects have not been universally demonstrated and the importance of such interactions for osteoporosis risk are not understood.

Perspective

Heritability studies have clearly demonstrated that genetic factors play a major role in determining phenotypic variance in bone mineral mass or BMD and variation in susceptibility to osteoporotic fracture. However, diet and other environmental factors make a sizeable contribution, and may be especially important for individuals with a particular genetic make-up. Since these factors are the most amenable to modification, optimisation of diet and lifestyle remains an attractive strategy for reducing fracture risk. The challenge for the research community is to define what is optimal.

References

American Society for Bone and Mineral Research (1999) *Primer of the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins.

Ames SK, Ellis KJ, Gunn SK, Copeland KC & Abrams SA (1999) Vitamin D receptor gene FokI polymorphism predicts calcium absorption and bone mineral density in children. *Journal of Bone and Mineral Research* **14**, 740–746.

Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, Nishisho T, Tonai T, Mori S & Takeda E (1997) A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. *Journal of Bone and Mineral Research* **12**, 915–921.

Arden N, Baker J, Hogg C, Baan K & Spector T (1996) The heritability of bone mineral density, ultrasound of the calcaneus and hip axis length: a study of postmenopausal twins. *Journal of Bone and Mineral Research* **11**, 530–534.

Arden NK & Spector TD (1997) Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study. *Journal of Bone and Mineral Research* **12**, 2076–2081.

Bass S, Delmas P, Pearce G, Hendrich E, Tabensky A & Seeman E (1999) The differing tempo of growth in bone size, mass, and density in girls is region specific. *Journal of Clinical Investigation* **104**, 795–804.

Beavan S, Prentice A, Yan L, Dibba B & Ralston S (1996) Differences in vitamin D receptor genotype and geographical variation in osteoporosis. *Lancet* **348**, 136–137.

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

Compston J (1993) Osteoporosis. In *The Management of Common Metabolic Bone Disorders*, pp. 29–62 [G Campbell, J Compston and A Crisp, editors]. Cambridge: Cambridge University Press.

Cooper C, Cawley M, Bhalla A, Egger P, Ring F, Morton L & Barker D (1995) Childhood growth, physical activity, and peak bone mass in women. *Journal of Bone and Mineral Research* **10**, 940–947.

Cooper C, Fall C, Egger P, Hobbs R, Eastell R & Barker D (1997) Growth in infancy and bone mass in later life. *Annals of the Rheumatic Diseases* **56**, 17–21.

Cooper GS & Umbach DM (1996) Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. *Journal of Bone and Mineral Research* **11**, 1841–1849.

Cumming RG & Nevitt MC (1997) Calcium for prevention of osteoporotic fractures in postmenopausal women. *Journal of Bone and Mineral Research* **12**, 1321–1329.

Cummings S, Black D, Nevitt M, Browner W, Cauley J, Ensrud K, Genant H, Palermo L, Scott J & Vogt T (1993) Bone density at various sites for prediction of hip fractures. *Lancet* **341**, 72–75.

Danielson ME, Cauley JA, Baker CE, Newman AB, Dorman JS, Towers JD & Kuller LH (1999) Familial resemblance of bone mineral density (BMD) and calcaneal ultrasound attenuation: the BMD in mothers and daughters study. *Journal of Bone and Mineral Research* **14**, 102–110.

Dawson-Hughes B, Harris S & Finneran S (1995) Calcium absorption on high and low calcium intakes in relation to vitamin D receptor genotype. *Journal of Clinical Endocrinology and Metabolism* **80**, 3657–3661.

Department of Health (1998) *Nutritional Aspects of Bone Health: With Particular Reference to Calcium and Vitamin D. Report of the Subgroup on Bone Health, Working Group on the Nutritional Status of the Population of the Committee on Medical Aspects of Food and Nutrition Policy*. London: The Stationery Office.

Dunger D, Ong K, Huxtable S, Sherriff A, Woods K, Ahmed M, Golding J, Pembrey M, Ring S, the ALSPAC Study team, Bennett S & Todd J (1998) Association of the *INS* VNTR with size at birth. *Nature Genetics* **19**, 98–100.

Ensrud KE, Stone K, Cauley JA, White C, Zmuda JM, Nguyen TV, Eisman JA & Cummings SR (1999) Vitamin D receptor gene polymorphisms and the risk of fractures in older women. *Journal of Bone and Mineral Research* **14**, 1637–1645.

Evans R, Marel G, Lancaster E, Kos S, Evans M & Wong S (1988) Bone mass is low in relatives of osteoporosis patients. *Annals of Internal Medicine* **109**, 870–873.

Ferrari S, Rizzoli R, Slosman D & Bonjour J-P (1998) Familial resemblance for bone mineral mass is expressed before puberty. *Journal of Clinical Endocrinology and Metabolism* **83**, 358–361.

Flicker L, Hopper J, Rodgers L, Kaymakci B, Green R & Wark J (1997) Bone density in elderly women: a twin study. *Journal of Bone and Mineral Research* **10**, 1607–1613.

Garn S (1970) *The Earlier Gain and Later Loss of Cortical Bone*. Springfield, IL: C.C. Thomas.

Greenfield EM & Goldberg VM (1997) Genetic determination of bone density. *Lancet* **350**, 1263–1264.

Hannan M, Felson D, Dawson-Hughes B, Tucker K, Cupples L, Wilson P & Kiel D (2000) Risk factors for longitudinal bone loss in elderly men and women: the Framingham Osteoporosis Study. *Journal of Bone and Mineral Research* **15**, 710–720.

Harris M, Nguyen TV, Howard GM, Kelly PJ & Eisman JA (1998) Genetic and environmental correlations between bone formation and bone mineral density: a twin study. *Bone* **22**, 141–145.

Hattersley A & Tooke J (1999) The fetal insulin hypothesis: an alternative explanation of the association of low birth weight with diabetes and vascular disease. *Lancet* **353**, 1789–1792.

Haussler MR, Whitfield GK, Haussler CA, Hsieh J-C, Thompson PD, Selznick SH, Dominguez CE & Jurutka PW (1998) The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *Journal of Bone and Mineral Research* **13**, 325–349.

Heaney RP, Barger-Lux MJ, Davies KM, Ryan RA, Johnson ML & Gong G (1997) Bone dimensional change with age: interactions of genetic, hormonal, and body size variables. *Osteoporosis International* **7**, 426–431.

Henderson N, Price R, Cole J, Gutteridge D & Bhagat C (1995) Bone density in young women is associated with body weight and muscle strength but not dietary intakes. *Journal of Bone and Mineral Research* **10**, 384–393.

Hopper J (1993) Variance components for statistical genetics: applications in medical research to characteristics related to human diseases and health. *Statistical Methods in Medical Research* **2**, 199–224.

Howard GM, Nguyen TV, Harris M, Kelly PJ & Eisman JA (1998) Genetic and environmental contributions to the association between quantitative ultrasound and bone mineral density measurements: a twin study. *Journal of Bone and Mineral Research* **13**, 1318–1327.

Kelly P, Hopper J, Macaskill G, Pocock N, Sambrook P & Eisman J (1991) Genetic factors in bone turnover. *Journal of Clinical Endocrinology and Metabolism* **72**, 808–814.

Kelly PJ, Nguyen T, Hopper J, Pocock N, Sambrook P & Eisman J (1993) Changes in axial bone density with age: a twin study. *Journal of Bone and Mineral Research* **8**, 11–17.

Krall EA & Dawson-Hughes B (1993) Heritable and life-style determinants of bone mineral density. *Journal of Bone and Mineral Research* **8**, 1–9.

Krall EA, Parry P, Lichter JB & Dawson-Hughes B (1995) Vitamin D receptor alleles and rates of bone loss: influences of years since menopause and calcium intake. *Journal of Bone and Mineral Research* **10**, 978–983.

Lutz J (1986) Bone mineral, serum calcium, and dietary intakes of mother/daughter pairs. *American Journal of Clinical Nutrition* **44**, 99–106.

Magarey AM, Boulton TJC & Chatterton BE (1999) Familial and environmental influences on bone growth from 11–17. *Acta Paediatrica* **88**, 1204–1210.

Marcus R, Feldman D & Kelsey J (editors) (1996) *Osteoporosis*. San Diego, CA: Academic Press.

Molgaard C, Thomsen BL, Prentice A, Cole TJ & Michaelsen KF (1997) Whole body bone mineral content in healthy children and adolescents. *Archives of Disease in Childhood* **76**, 9–15.

Morrison N, Qi J, Tokita A, Kelly P, Croft L, Nguyen T, Sambrook P & Eisman J (1997) Prediction of bone density by vitamin D receptor alleles: corrections. *Nature* **387**, 106.

Morrison N, Yeoman R, Kelly P & Eisman J (1992) Contribution of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphisms and circulating osteocalcin. *Proceedings of the National Academy of Sciences USA* **89**, 6665–6669.

Morrison NA, Cheng J, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN & Eisman JA (1994) Prediction of bone density from vitamin D receptor alleles. *Nature* **367**, 284–287.

Nguyen T, Howard G, Kelly P & Eisman J (1998) Bone mass, lean mass, and fat mass: same genes or same environments? *American Journal of Epidemiology* **147**, 3–16.

Nguyen TV, Blangero J & Eisman JA (2000) Genetic epidemiological approaches to the search for osteoporosis genes. *Journal of Bone and Mineral Research* **15**, 392–400.

Nilsson A, Ohlsson C, Isaksson O, Lindahl A & Isgaard J (1994) The hormonal regulation of longitudinal bone growth. *European Journal of Clinical Nutrition* **48**, Suppl. 1, S150–S160.

Nordstrom P & Lorentzon R (1999) Influence of heredity and environment on bone density in adolescent boys: a parent-offspring study. *Osteoporosis International* **10**, 271–277.

Ong K, Golding J, Todd J & Dunger D (1999) Genetic influences on fetal growth. In *Fetal Programming: Influences on Development and Disease in Later Life*, pp. 85–96 [P O'Brien, T Wheeler and D Barker, editors]. London: RCOG Press.

Parsons TJ, Prentice A, Smith EA, Cole TJ & Compston JE (1996) Bone mineral mass consolidation in young British adults. *Journal of Bone and Mineral Research* **11**, 264–274.

Pocock N, Eisman J, Hopper J, Yeates M, Sambrook P & Eberl S (1987) Genetic determinants of bone mass in adults. *Journal of Clinical Investigation* **80**, 706–710.

Pollitzer WS & Anderson JJB (1989) Ethnic and genetic differences in bone mass: a review with a hereditary vs environmental perspective. *American Journal of Clinical Nutrition* **50**, 1244–1259.

Pols HAP, Uitterlinden AG & Leeuwen JPTMv (1998) How about vitamin D receptor polymorphisms? *Osteoporosis International* **8**, Suppl. 2, S20–S23.

Prentice A (1997) Is nutrition important in osteoporosis? *Proceedings of the Nutrition Society* **56**, 357–367.

Prentice A & Bates CJ (1994) Adequacy of dietary mineral supply for human bone growth and mineralisation. *European Journal of Clinical Nutrition* **48**, Suppl. 1, S161–S177.

Prentice A, Parsons TJ & Cole TJ (1994) Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants. *American Journal of Clinical Nutrition* **60**, 837–842.

Price J, Oyajobi B & Russell R (1994) The cell biology of bone growth. *European Journal of Clinical Nutrition* **48**, Suppl. 1, S131–S149.

Ralston SH (1998) Do genetic markers aid in risk assessment? *Osteoporosis International* **8**, Suppl. 1, S37–S42.

Ralston SH (1999) The genetics of osteoporosis. *Bone* **25**, 85–86.

Roberts D, Billewicz W & McGregor I (1978) Heritability of stature in a West African population. *Annals of Human Genetics* **42**, 15–24.

Saupe J, Shearer MJ & Kohlmeier M (1993) Phylloquinone transport and its influence on gamma-carboxyglutamate residues of osteocalcin in patients on maintenance dialysis. *American Journal of Clinical Nutrition* **58**, 204–208.

Schiessl H, Frost H & Jee W (1998) Estrogen and bone-muscle strength and mass relationships. *Bone* **22**, 1–6.

Seeman E & Hopper J (1997) Genetic and environmental components of the populations variance in bone density. *Osteoporosis International* **7**, Suppl. 3, S10–S16.

Seeman E, Hopper J, Bach L, Cooper M, Parkinson E, McKay J & Jerums G (1989) Reduced bone mass in daughters of women with osteoporosis. *New England Journal of Medicine* **320**, 554–558.

Seeman E, Hopper J, Young N, Formica C, Goss P & Tsalamandris C (1996) Do genetic factors explain the association of muscle strength, lean mass and bone density? A twin study. *American Journal of Physiology* **270**, E320–E327.

Seeman E, Tsalamandris C, Formica C, Hopper J & McKay J (1994) Reduced femoral neck bone density in the daughters of women with hip fractures: the role of low peak bone density in the pathogensis of osteoporosis. *Journal of Bone and Mineral Research* **9**, 739–743.

Slemenda CW, Christian JC, Williams CJ, Norton JA & Johnston CC (1991) Genetic determinants of bone mass in adult women: a reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. *Journal of Bone and Mineral Research* **6**, 561–567.

Smith D, Nance W, Won Kang W, Christian J & Johnston C (1973) Genetic factors in determining bone mass. *Journal of Clinical Investigation* **52**, 2800–2808.

Soroko S, Barrett-Connor E, Edelstein S & Kritz-Silverstein D (1994) Family history of osteoporosis and bone mineral density at the axial skeleton: The Rancho Bernardo Study. *Journal of Bone and Mineral Research* **9**, 739–743.

Tao C, Yu T, Garnett S, Briody J, Knight J, Woodhead H & Cowell CT (1998) Vitamin D receptor alleles predict growth and bone density in girls. *Archives of Disease in Childhood* **79**, 488–494.

Tylavsky FA, Bortz AD, Hancock RL & Anderson JJB (1989) Familial resemblance of radial bone mass between pre-menopausal mothers and their college-age daughters. *Calcified Tissue International* **45**, 265–272.

Widdowson EM & Dickerson JWT (1964) Chemical composition of the body. In *Mineral Metabolism*, pp. 1–247 [CL Cornar and F Bronner, editors]. New York: Academic Press.

Young D, Hopper J, Nowson C, Green R, Sherwin A, Kaymakci B, Smid M, Guest C, Larkins R & Wark J (1995) Determinants of bone mass in 10- to 26-year-old females: a twin study. *Journal of Bone and Mineral Research* **10**, 558–567.