

Nutritional influences on bone mass

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Bone is composed of an organic matrix of type I collagen associated with non-collagenous proteins and a large mineral component consisting of calcium hydroxyapatite. To date, measurement of bone mass only takes into account the mineral component of bone, while recognizing that the organic matrix is important in providing both structure and strength. The present paper will concentrate on the influence of nutrition on bone mass, examining initially the effects of bone mass with ageing, defining the principal disease of bone mass (i.e. osteoporosis), considering the genetic and environmental factors which affect bone mass and summarizing the methods of bone health assessment. The review will then focus on the nutritional factors (from both dietary sources and in the form of supplementation) which may influence bone mass and fracture rates. Finally, examination of how our knowledge of nutrition factors could be used to target lifestyle changes in individuals at risk of future osteoporosis will be discussed.

BONE MASS AND OSTEOPOROSIS

Bone mass in later life depends on the peak bone mass achieved during growth and the rate of subsequent age-related bone loss. Bone mass is gained throughout childhood and accelerates during the prepubertal growth spurt (Bonjour *et al.* 1991). From the age of 20 years until approximately 40 years, bone mass is stable in both sexes, with a linear rate of loss in men starting in the fifth decade but an accelerated rate of loss in women just after the menopause (Kroger *et al.* 1992). This accelerated rate of loss seen in women (which occurs especially at trabecular-rich sites such as the spine or wrist), when associated with a low attainment of peak bone mass, leads to excessive risk of future fracture.

The condition of osteoporosis has been defined as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture (Consensus Development Conference, 1993). The difficulty in using this definition in clinical practice has led to a Working Party of the World Health Organization redefining osteoporosis according to bone mass, at least for women. Osteoporosis has been defined as a bone mineral content or bone mineral density (BMD) more than 2.5 standard deviations below the young normal mean, and established osteoporosis as the same mass definition but associated with a fragility fracture (World Health Organization, 1994).

GENETIC AND ENVIRONMENTAL INFLUENCES ON BONE MASS

It is beyond the scope of the present article to review the pathogenesis of osteoporosis, but it is clear that predisposition to osteoporotic fracture is related first to peak bone mass and second to the rate of bone loss. Both peak bone mass and rates of loss are determined by a

combination of genetic and environmental factors and recently these have been elegantly reviewed (Ralston, 1996). It is considered that up to 75 % of the variation in bone mass in the elderly is influenced by genetic factors (Flicker *et al.* 1995), leaving a relatively limited role for environmental associates including nutrition. However, some genetic markers may influence the effectiveness of nutritional factors in controlling bone loss. This has been demonstrated in relation to the vitamin D-receptor polymorphisms which may influence Ca-related bone loss in the elderly (Ferrari *et al.* 1995).

ASSESSMENT OF BONE MASS

As osteoporosis has been defined according to the amount of bone present in the skeleton, it is important to have methods available for accurate and precise assessment. The most commonly used technique to date is dual-energy X-ray absorptiometry (DXA) which uses a tiny dose of X-rays (Miller *et al.* 1995). DXA can be used to define risk of future fracture, diagnose low bone mass or osteoporosis and assess the response to treatment for the disease.

The last decade has seen the development of a new and exciting technique known as broadband ultrasound attenuation (BUA). The method has great potential since it is relatively inexpensive, highly portable and uses no harmful radiation (Heaney & Kanis, 1996). It can be used to predict elderly patients at risk of hip fractures, but does not measure BMD directly and, therefore, cannot be used at present to define osteoporosis. There are persisting claims that it may measure structural components which are not assessed by DXA (Njeh *et al.* 1997), but further research is required.

In assessing the nutritional influences on bone mass it is particularly pertinent to know that bone mineral 'density' corrects for projected bone area and, therefore, is not a true density measurement. Many studies have demonstrated that this 'areal' BMD does not adequately correct for the influences of body size and some studies have indicated that BMD remains significantly correlated with projected bone area (Prentice *et al.* 1994). Although methods have been described to estimate volumetric bone density, there seems no major advantage to this technique in terms of the discriminatory value of bone mass in patients with fractures or in precision measurements (Mazess *et al.* 1994).

NUTRITIONAL INFLUENCES ON BONE MASS

A large number of macro- and micronutrients have been considered to have some effect on bone health. It is not possible to cover all these nutrients in the following review and, thus, attention will be focused on Ca, protein, fibre, K and trace elements.

Calcium

Since Ca is the most important mineral constituent of the skeleton, its role in the regulation of bone mass has been the centre of much controversy (Kanis & Passmore, 1989*a,b*; Nordin & Heaney, 1990). However, in a meta-analysis of all studies up to 1988 relating Ca intake and bone status, it was concluded that the evidence supported a positive relationship between the two variables (Cummings, 1990).

The association between Ca and bone health will be reviewed as follows: (1) Ca intake during skeletal growth; (2) Ca intake in the premenopausal years; (3) relationship of Ca intake to bone loss (early and late post-menopausal); (4) intake of Ca and risk of fracture.

Calcium intake during skeletal growth. Studies at the beginning of the century showed that children who consumed milk grew taller compared with subjects without milk supplementation (Orr, 1928; Leighton & Clark, 1929). Although BMD assessment was not available at that time, there is now accumulating evidence for the benefits of Ca intake on bone mass during childhood (Lee *et al.* 1993), adolescence (Matkovic *et al.* 1990) and early adulthood in British (Fehily *et al.* 1992), Finnish (Valimaki *et al.* 1994), Chinese (Ho *et al.* 1994) and American women (Kanders *et al.* 1988; Recker *et al.* 1988). There are also data to show that a high past intake of milk is beneficial to bone health in premenopausal (New *et al.* 1997), post-menopausal (Sandler *et al.* 1985) and elderly women (Murphy *et al.* 1994).

Ca supplementation has been shown also to positively influence bone mass during skeletal growth in both children (Johnston *et al.* 1992) and young girls (Lloyd *et al.* 1993), and a similar effect has been reported with milk supplementation in British adolescents (Cadogan *et al.* 1995). In the 3-year study of identical twins by Johnston *et al.* (1992), skeletal density increased in children receiving Ca supplements of 700 mg/d in addition to their normal diets. However, in a follow-up of these subjects 2 years later, the effect of Ca intake had all but disappeared, raising the question of whether the effect is ephemeral (Slemenda *et al.* 1993).

There is increasing evidence to suggest that Ca is a threshold nutrient. While sufficient exogenous Ca must be present to sustain density during growth and maintain skeletal mass in later life, additional Ca above the threshold may not necessarily produce more bone gain than that which is predestined by both genetic predisposition and levels of mechanical loading (Matkovic, 1991). Analysis of 487 Ca balance studies indicated the presence of a balance threshold at all stages of growth, from infancy up through the skeletal consolidation years (Matkovic & Heaney, 1992). The threshold values were found to be 1400 mg/d in children aged 2–8 years, 1480 mg/d in adolescents aged 9–17 years and 960 mg/d in young adults aged 18–30 years. These values are higher than the recommended dietary allowance (RDA; National Research Council, 1989) and dietary reference values (DRV; Department of Health, 1991) for the ages concerned both in the USA and UK. A recent Canadian review has also led to the conclusion that recommended dietary intakes of Ca are too low (Murray, 1996).

Calcium intake in the premenopausal years. Only two studies have shown Ca supplementation to be of benefit in premenopausal women. Baran *et al.* (1990) found no bone loss in women supplemented with dairy products, compared with increased loss in the unsupplemented group. However, the difference was detectable in the 3rd year, at which time there was a 37 % withdrawal rate. More recently, Rico *et al.* (1994) found a positive effect of supplementation of calcium pidolate on bone mass.

There have been a number of studies examining the relationship between dietary Ca intake and bone mass in premenopausal women. Although some have reported a significant association between Ca intake and BMD in premenopausal women, including studies in European (Matkovic *et al.* 1979), British (Ramsdale *et al.* 1994), and Chinese women (Hu *et al.* 1993), other groups, including our own, have found there to be no relationship (McCulloch *et al.* 1990; Valimaki *et al.* 1994; New *et al.* 1997).

There are several reasons which may help to explain this apparent discrepancy. Those studies which report a positive relationship between Ca intake and bone mass fail to

comment on the fact that in subjects with higher Ca intakes, energy intakes were also higher and yet body weight was not significantly different, thus suggesting increased physical activity. Furthermore, dietary intake has often been assessed using a method which is unreliable and unrepresentative of habitual food intake. This unreliability is confirmed by reported energy intakes which are extremely low ($< 1.2 \times \text{BMR}$), indicating the likelihood of under-reporting and, thus, potentially biased data.

Relationship between calcium intake and bone loss. Virtually all published studies show little or no effect of dietary or supplementary Ca during the 5 years following the menopause (Ettinger *et al.* 1987; Riggs *et al.* 1987; Riis *et al.* 1987; Stevenson *et al.* 1988; Dawson-Hughes *et al.* 1990). Elders *et al.* (1991) showed that supplementation with 1000 and 2000 mg elemental Ca/d retarded bone loss in the first year of supplementation, but not in the second year. In the study by Dawson-Hughes *et al.* (1990) women who were less than 5 years post-menopause, had rapid bone loss not affected by Ca supplementation (500 mg/d). However, in women who had been post-menopausal for 6 years or more, with a daily intake of Ca of less than 400 mg/d, bone loss was significantly reduced. High-dose supplementation (1000 mg/d) may have a benefit on total and site-specific bone mass in those 10 years from the menopause, even when dietary intake of Ca is satisfactory (Reid *et al.* 1993). This supplementation effect may last for as long as 4 years and result in fewer fractures, although bone loss is not entirely prevented (Reid *et al.* 1995). Positive influences of dietary Ca on bone density (Andon *et al.* 1991) and supplementary Ca on bone loss (Smith *et al.* 1989; Nelson *et al.* 1991; Prince *et al.* 1991) have also been reported in other groups of older women.

Intake of calcium and risk of fracture. The study by Matkovic *et al.* (1979) was the first to demonstrate anti-fracture efficacy for high-Ca diets, with a reduction in risk at the hip, but not at the wrist. Several epidemiological studies have since been reported with conflicting results. Holbrook *et al.* (1988) in a 14-year longitudinal study found that hip fracture rates were lower in individuals ingesting high-Ca diets and similar findings were reported in the Hong Kong population (Lau *et al.* 1988). In the UK, Cooper *et al.* (1988) compared physical activity and Ca intakes of 300 elderly men and women who had sustained hip fractures with 600 age- and sex-matched controls. Ca intake was not related to the risk of fracture in women, but men with an intake greater than 1000 mg/d had lower risks. In a further UK study, Wickham *et al.* (1989) also found no evidence for a relationship between Ca intake and risk of hip fracture.

Using fracture itself as the end point, Chapuy *et al.* (1992) in a large and well-controlled study, showed substantial reductions in fracture rates in a large group of institutionalized elderly given supplements of Ca and cholecalciferol. Protection became apparent after 6–12 months of treatment, and by 18 months, fractures were reduced by more than 30 % in the treated individuals. Similar results were found when an annual injection of cholecalciferol was given (Heikinheimo *et al.* 1992), but no differences were seen with cholecalciferol without Ca (Lips *et al.* 1996). In the most recent study, Ca supplementation in vitamin D-replete elderly subjects was found to reduce fracture rates (Chevalley *et al.* 1994).

In summary, while differences in the findings of studies remain, there is some agreement that Ca intake (and indeed supplementation) is beneficial during peak bone mass development, and has some effect at slowing bone loss in late post-menopausal women, (particularly if Ca intakes are low). There is also growing evidence that it may even help prevent hip fracture.

Protein

Increased protein intake has been associated with an increase in urinary Ca excretion, thus shifting Ca balance in a negative direction. With protein intakes below 175 g/d, the relationship between dietary protein and urinary Ca has been shown to be linear, such that for each 50 g increment of protein consumed, an extra 60 mg urinary Ca is lost (Kersetter & Allen, 1989).

The decrease in Ca absorption which occurs with increased protein intake is related to the acid generated from protein metabolism. Oxidation of the S amino acids, methionine and cysteine, yields inorganic sulfate and two H ions. Metabolic acidosis is associated with elevated urinary Ca, and the addition of a base reduces urinary Ca losses. Approximately half the urinary Ca can be attributed to the S amino acids and acid load, and the elevated glomerular filtration rate immediately following a high-protein meal is likely to contribute to the other half. It has been shown that serum bicarbonate remains normal during H ion retention, and thus in order to respond to the acid load imposed by protein intake, it is possible that bone is buffered to maintain the acid–base balance (Lutz, 1984).

Thus, populations which consistently consume high levels of animal protein, should, in theory at least, have a higher prevalence of low-BMD and osteoporotic-related fractures, and increased bone resorption. Hegsted (1986) noted high protein intakes were associated with a higher incidence of hip fractures across several nations, and in a recent meta-analysis of thirty-four publications in sixteen countries, a strong positive association existed between animal protein intake and age-adjusted female fracture rates (Abelow, 1992). However, the results of studies comparing omnivorous diets with lacto-vegetarian diets are conflicting. Some researchers have found high bone density in vegetarian women consuming less protein (Marsh *et al.* 1980, 1988), while others have concluded differently (Tylavsky & Anderson, 1988; Hunt *et al.* 1989; Tesar *et al.* 1992). In a recent study, no differences were found in rate of bone loss between elderly lacto-ovo-vegetarians and omnivores (Reed *et al.* 1994). Furthermore, a recent population-based study indicated that dietary intake of protein was positively correlated with bone mass at the lumbar spine, hip and radius, although these associations were reduced after age adjustment and were only present in premenopausal women (Cooper *et al.* 1996).

The effect of dietary protein insufficiency on bone health has received much less attention, but there is some evidence to show that hip-fracture patients are deficient in protein and energy (Mitchell & Lipschitz, 1982) and may benefit from supplementation (Delmi *et al.* 1990).

In summary, while it is clear that high protein intakes affect Ca balance and possibly acid–base status, long-term influences of high intakes of protein, outside the experimental setting, have yet to prove substantially that excess protein intake is detrimental to bone health.

Fibre

Increased fibre intake (using wheat bran as the fibre source) has been associated with a decrease in Ca absorption, which can be summarized as a decrease in absorption of about 20–30 % when going from an intake of 0.1 to approximately 30 g bran fibre/d (Heaney *et al.* 1991). Fibre intake has also been associated with increased urinary Ca excretion and lower oestradiol levels in pre- and post-menopausal women (Barbosa *et al.* 1990; Feng *et al.* 1993). This reduction in hormone levels may be due to the interruption in the enterohepatic circulation of sex hormones. Such findings, for both reduced Ca absorption and

decreased oestradiol levels has led to the assumption that increased intakes of fibre may have a negative effect on bone mass.

There is very little evidence for such an effect. Negative correlations between trabecular bone density (Leuenberger *et al.* 1989) and fibre intake have been reported, while others have found no association (Yano *et al.* 1985). Increased bone loss with high fibre intake has been reported by our group, but only in diets which are weight reducing (Avenell *et al.* 1994).

The long-term lowering effects of high-fibre diets on circulating oestradiol levels and bone health has not yet been established. However, high Ca absorption from whole-wheat bread has been reported, similar to that measured for milk containing a comparable Ca load (Heaney *et al.* 1991). Good absorption for Ca has also been obtained from low-phytate foods such as kale (*Brassica oleracea acephala*) and soyabeans, (which contain large amounts of fibre) compared with a low Ca absorption from vegetables high in oxalate such as spinach (*Spinacia oleracea*; Heaney *et al.* 1988; Heaney & Weaver, 1990). Wisker *et al.* (1991) found that large additions of low-phytate barley-fibre concentrate did not interfere with Ca absorption in normal adults. The high-fibre diet associations with reduced oestradiol levels have also only been found with wheat-bran fibre. Thus, the effect on Ca absorption may be due to phytate (which is present in substantial amounts in wheat bran), rather than the fibre intake per se (C. M. Weaver, personal communication). High fibre intakes from a mixed diet, therefore, may not be detrimental to bone health, which may help to explain the positive relationship seen between spine BMD and fibre intake in our own epidemiological study (New *et al.* 1997).

Potassium

K deserves special consideration as there is growing evidence for beneficial effects of KHCO_3 on mineral balance and skeletal metabolism. As noted by Wachman & Bernstein (1968), the skeleton is not only a labile reservoir of Ca responsive to the mechanisms which maintain ionic Ca, but it also contains a reservoir of alkaline salts of Ca which, in turn, provide a source of labile base. This can be mobilized to react to both blood pH and plasma bicarbonate concentrations. The failure to maintain adequate acid-base homeostasis in adults has been proposed as the main reason for the progressive decline in bone mass which occurs with age (Wachman & Bernstein, 1968), excepting of course the rapid immediately post-menopausal loss mediated by oestrogen lack. Such bone loss may be due to the lifelong mobilization of skeletal calcium salts to balance the endogenous acid generated from foods such as meat.

KHCO_3 has been shown to reduce urinary Ca excretion and improve Ca balance in healthy young adults (Lemann *et al.* 1991) and has also been shown to reduce bone resorption and increase the rate of bone formation in post-menopausal women (Sebastian *et al.* 1994). More recently, positive associations have been reported between dietary intakes of K and bone mass both by our group (New *et al.* 1997, 1998) and others (Michaelsson *et al.* 1995), and its role in bone health certainly warrants further attention.

Trace elements

Zn, Cu and Mn are known to be essential metallic cofactors for enzymes involved in the synthesis of various bone matrix constituents, but their role in bone health is unclear. Positive associations between dietary intake of Zn and bone mass have been reported in women (Freudenheim *et al.* 1986; Angus *et al.* 1988), and work by Strause *et al.* (1994) has

shown that bone loss in Ca-supplemented older post-menopausal women can be further arrested by concomitant increases in trace mineral intake. Furthermore, these trace elements (and vitamin C) may also function in their antioxidant capacity and, thus, high intakes may be important if the connective tissue of bone is a target for free-radical damage. This is an area which has received relatively little attention in the literature and warrants further study.

Awareness of the possible importance of Mg to bone health is also increasing. Several studies have noted a positive relationship between Mg intake and bone density. Cancellous bone of osteoporotic women has been shown to have a reduced Mg content compared with healthy bone from age-matched controls (Manicourt *et al.* 1981). Although Mg deficiency in animals causes increased bone resorption, its role in human bone metabolism is unclear and further studies are required to establish the optimal Mg vehicle, dosage and duration of treatment.

PREVENTION OF OSTEOPOROSIS BY LIFESTYLE ADVICE

The options for using the information on the relationship between bone mass, fracture risk and nutritional factors are to use these to influence lifestyle changes in the entire population or to target preventative therapy in those at most risk of fracture. Prevention might be possible by increasing dietary Ca, vitamin D and possibly K, Mg and vitamin K. These dietary changes would almost certainly have to be associated with a general increase in weight-bearing exercise.

There are likely to be considerable costs in encouraging patients to take this lifestyle advice. In a randomized controlled trial of screening for osteoporosis, our own group were able to improve uptake of hormone-replacement therapy in those at risk of osteoporosis but were unable to affect the numbers of smokers or the exercise taken by those found to have low bone mass (Torgerson *et al.* 1997). It seems likely, therefore, that considerable effort would require to be expended on persuading women to alter their lifestyle, even when selected by the finding of a major risk factor, such as the presence of a genetic marker, presence of environmental risk factors including nutrition or low bone mass. Targeting of lifestyle advice may also be age-specific and may be particularly relevant in childhood and the elderly.

CONCLUDING REMARKS

If dietary manipulation is to be used as a preventative strategy, substantial reinforcement will be required and the costs of achieving such changes in the nation's diet will not be negligible. Targeting of advice, therefore, is likely to be necessary and primary intervention research is required to prove this hypothesis.

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