

The Summer Meeting of the Nutrition Society, hosted by the Irish Section, was held at the University of Ulster, Coleraine on 16–19 July 2007

Symposium on ‘Diet and bone health’

Importance of calcium, vitamin D and vitamin K for osteoporosis prevention and treatment

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Throughout the life cycle the skeleton requires optimum development and maintenance of its integrity to prevent fracture. Bones break because the loads placed on them exceed the ability of the bone to absorb the energy involved. It is now estimated that one in three women and one in twelve men aged >55 years will suffer from osteoporosis in their lifetime and at a cost in the UK of $>£1.7 \times 10^9$ per year. The pathogenesis of osteoporosis is multifactorial. Both the development of peak bone mass and the rate of bone loss are determined by key endogenous and exogenous factors. Ca supplements appear to be effective in reducing bone loss in women late post menopause (>5 years post menopause), particularly in those with low habitual Ca intake (<400 mg/d). In women early post menopause (<5 years post menopause) who are not vitamin D deficient, Ca supplementation has little effect on bone mineral density. However, supplementation with vitamin D and Ca has been shown to reduce fracture rates in the institutionalised elderly, but there remains controversy as to whether supplementation is effective in reducing fracture in free-living populations. Re-defining vitamin D requirements in the UK is needed since there is evidence of extensive hypovitaminosis D in the UK. Low vitamin D status is associated with an increased risk of falling and a variety of other health outcomes and is an area that requires urgent attention. The role of other micronutrients on bone remains to be fully defined, although there are promising data in the literature for a clear link between vitamin K nutrition and skeletal integrity, including fracture reduction.

Osteoporosis: Fracture: Vitamin D: Calcium: Vitamin K

Bones break because the loads placed on them exceed their ability to absorb the energy involved⁽¹⁾. This outcome is likely to be the result of a number of factors including, but not limited to: a reduction in bone mass; a change in the distribution of bone; loss of cancellous or cortical micro-architecture; an accumulation of damaged bone; a change in the material properties of the remaining bone⁽²⁾.

Bone is a living tissue. There is a continuous cycle of active bone formation (through the activity of osteocytes and osteoblasts) and bone resorption (involving osteoclasts). Fig. 1 shows the sequence of events in the bone remodelling sequence. Osteoclasts are attracted to a quiescent bone surface and then excavate an erosion cavity. Mononuclear cells smooth off the erosion cavity,

which is a subsequent site for the attraction of osteoblasts that synthesise an osteoid matrix. Continuous new bone matrix synthesis is followed by calcification of the newly-formed bone. When complete, lining cells once more overlie the trabecular surface⁽³⁾.

Osteoporosis is defined as a metabolic bone disease ‘characterised by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk’^(4,5). There is a normal mineral:collagen, unlike that of other metabolic bone diseases such as osteomalacia (which is characterised by deficiencies of mineral relative to collagen)⁽⁶⁾. An example of osteoporotic and normal bone is shown in Fig. 2.

Abbreviations: BMD, bone mineral density; 25 OHD, 25-hydroxyvitamin D.

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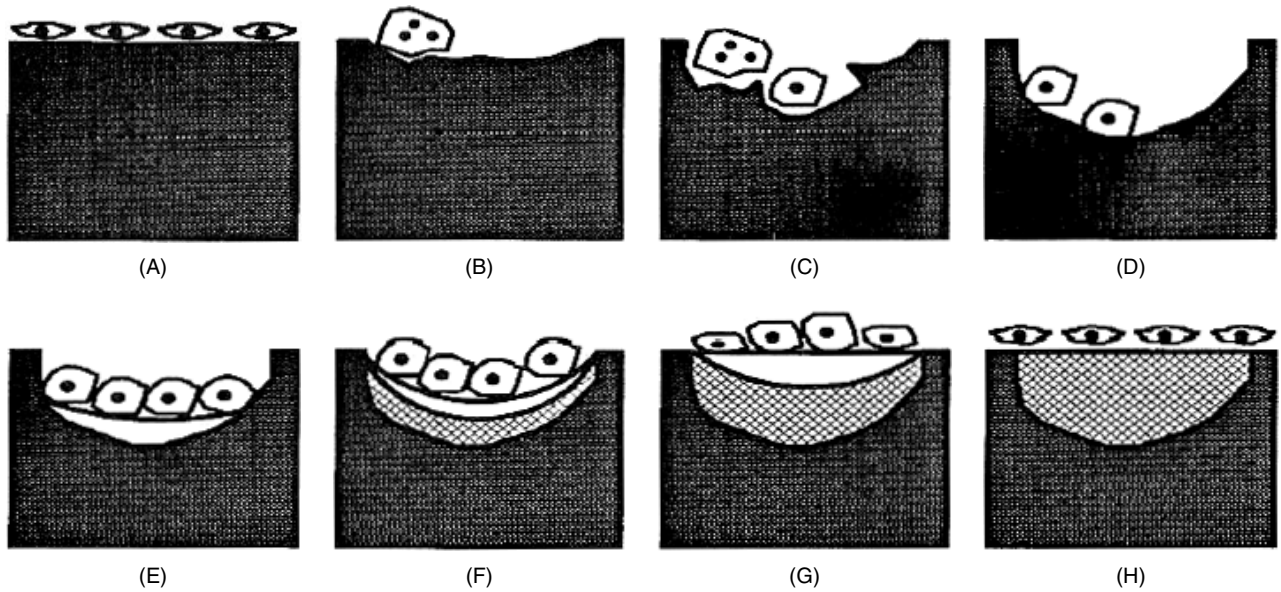


Fig. 1. Steps in the remodelling sequence of cancellous bone. Osteoclasts are attracted to a quiescent bone surface (A) and then excavate an erosion cavity (B, C). Mononuclear cells smooth off the erosion cavity (D), which is a subsequent site for the attraction of osteoblasts that synthesise an osteoid matrix (E). Continuous new bone matrix synthesis (F) is followed by calcification (G) of the newly-formed bone. When complete, lining cells once more overlie the trabecular surface (H). (From the National Osteoporosis Society⁽¹⁴⁾; reproduced with permission.)

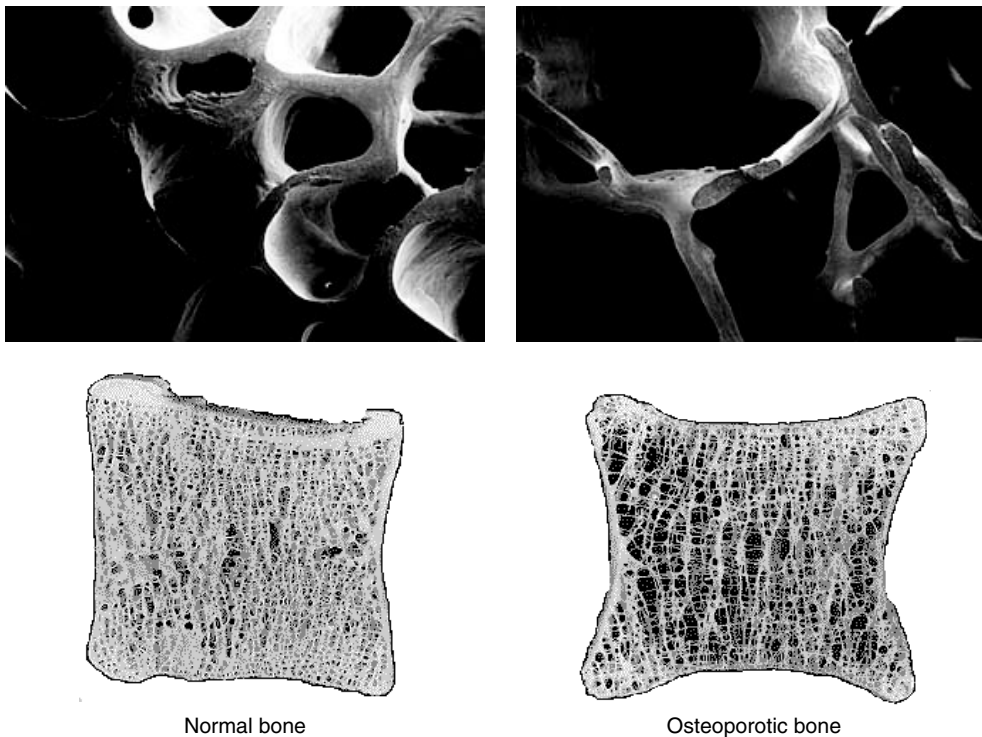


Fig. 2. Examples of normal and osteoporotic bone under the microscope. (From Eastell⁽³⁾; reproduced with permission.)

Public health impact of poor bone health

Globally, it is estimated that one in three women and one in twelve men aged >50 years will suffer from osteoporosis in their lifetime, which roughly equates to three million individuals⁽⁷⁾. There are >230 000 osteoporotic

fractures occurring every year in the UK at a cost of >£1.7 × 10⁹ per year. In Europe the cost of treating osteoporotic fractures is an important contributor to the financial implications of osteoporosis generally (it is estimated that €13.9 × 10⁹ is the expected annual total cost relating to osteoporosis)⁽⁸⁾. Specifically, hip fractures

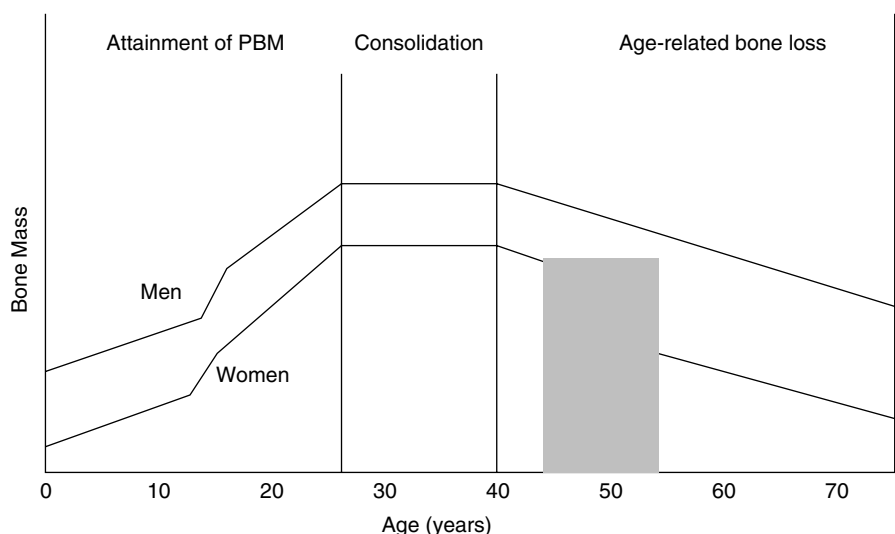


Fig. 3. Changes in bone mass during the life cycle. Critical times are: (1) attainment of peak bone mass (PBM; 0–28 years of age, with pubertal years being particularly crucial); (2) menopause (■); during the menopause and ≤ 10 years post menopause it is estimated that 1–2% of bone is lost per year; (3) age-related bone loss (a low bone mineral density threshold increases osteoporosis fracture risk).

account for more than one-third of the total cost of osteoporosis and are a reflection of the inpatient (hospital) and outpatient (nursing home) care⁽⁹⁾. Furthermore, the WHO has projected a rise in osteoporotic fractures from 1.66×10^6 hip fractures in 1990 to 6.26×10^6 in the 2050, which suggests that the future economic impact of osteoporosis will be enormous⁽¹⁰⁾.

Changes in bone mass with ageing

Considerable changes in skeletal mass occur during the life cycle. Adult bone health is predominantly determined by three key factors: the maximum attainment of peak bone mass, which is achieved during growth and early adulthood; maintaining bone mass in adulthood; reducing the rate of bone loss with advancing age, with the menopausal years being a time of considerable concern for women (Fig. 3)⁽¹¹⁾. Both peak bone mass attainment in the younger population and the rate of bone loss in post-menopausal women and the elderly are determined by key endogenous and exogenous factors, i.e. a combination of genetic, endocrine, mechanical and nutritional factors⁽¹²⁾, with evidence of extensive interactions within and between these groups.

Calcium nutrition: important concepts

Ca is the most abundant mineral in the body⁽¹³⁾. Approximately 1.2 kg (equivalent to about 300 mmol) is contained within the human body, with 99% of this Ca being located within the bones and teeth. Ca is also located in body fluids and soft tissues. It has two key roles: (1) supporting structural integrity; (2) regulating metabolic function⁽¹⁴⁾. Ca is essential for: cellular structure; intercellular and intracellular metabolic function; signal transmission; muscle contractions, including heart muscle; nerve function; activities of enzymes; normal clotting of blood. There

is no functional marker of Ca status, since its role in normal blood clotting takes priority and hence plasma Ca is maintained within very narrow limits (Fig. 4)⁽¹⁵⁾.

Ca metabolism

Ca absorption occurs predominantly in the jejunum and also in the ileum and colon. Uptake occurs by active transport and simple passive diffusion. At low Ca intakes active transport predominates, but as intakes increase more is absorbed by non-specific pathways⁽¹⁶⁾. The metabolite of vitamin D (1,25-dihydroxycholecalciferol) stimulates Ca transport across the intestinal cells by inducing the production of a Ca-binding protein. This process occurs within the villus cells through the normal process of receptor binding, DNA interaction and messenger RNA production. Hence, vitamin D is critical for effective Ca absorption⁽¹⁷⁾.

Ca is lost mainly through renal excretion but there are data to also support losses occurring via faeces, sweat, skin and hair, as well as nails. Ca enters the gut via the bile, which is known to be relatively rich in Ca. It also enters the gut in pancreatic secretions and as part of desquamated cells from the mucosal lining. It may also be reabsorbed from the ileum and colon. As a result of the endogenous secretion the net absorption is less than gross dietary absorption by approximately 100 mg. Plasma Ca is tightly maintained at a level of 90–105 mg/l, of which approximately 50% is ionised. The plasma ionised Ca compartment is maintained by a combination of humeral factors that regulate intestinal absorption, renal loss and deposition or mobilisation of Ca from bone⁽¹⁸⁾.

Urinary Ca excretion

Approximately 97% of the filtered Ca load is re-absorbed by the renal tubules and the remaining 3%, which is

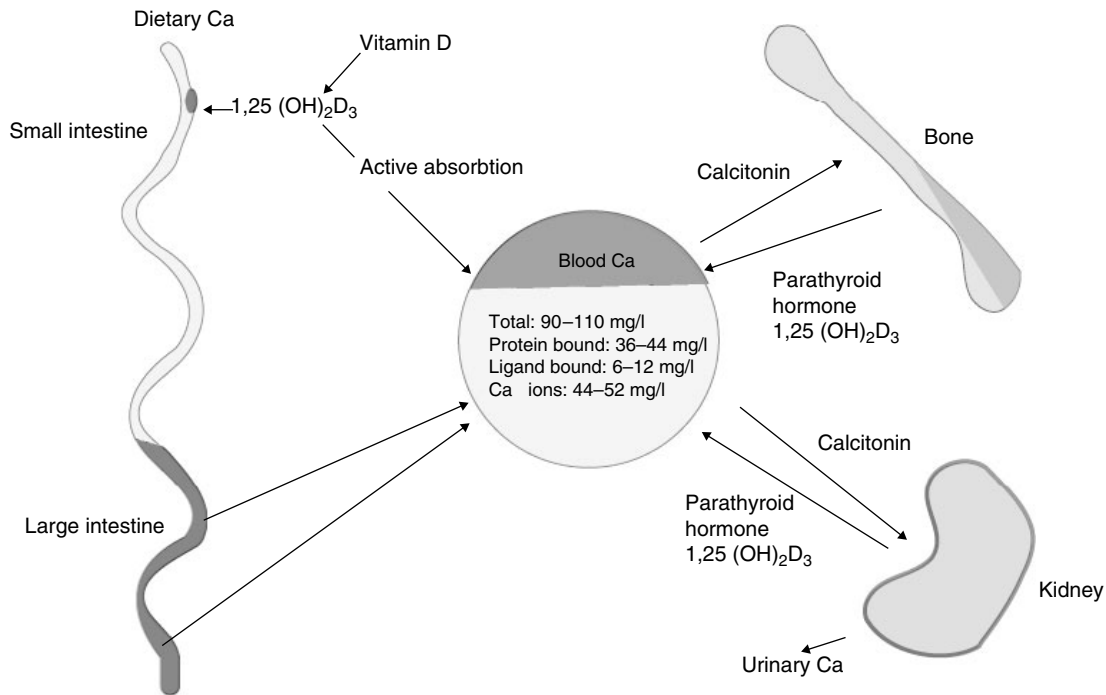


Fig. 4. Regulation of calcium levels in blood and tissue. 1,25 (OH)₂D₃, 1,25-dihydroxycholecalciferol. (From Smith⁽¹²⁾; reproduced with permission.)

excreted in urine, represents the obligatory loss. The major determinant of urinary Ca excretion is dietary Ca intake. Urinary Ca excretion is higher when protein and Na intakes are high than when intakes of these two nutrients are low. The effects of a high protein intake on Ca excretion may be partly offset by higher P intake. Urinary Ca excretion decreases in old age with glomerular filtration rate. Also, intestinal absorption declines as the result of a reduction in the efficiency of parathyroid hormone and vitamin D metabolism. Increased urinary Ca in women at the menopause reflects the increased mobilisation of bone Ca because of oestrogen deficiency⁽¹⁹⁾.

Control of Ca balance

An adult on a normal mixed diet is usually in a state of equilibrium, i.e. the amount lost in the faeces and urine is approximately equal to the amount present in the food. In growing children the body is normally in positive balance. Ca is steadily maintained for the formation of new bone. When the need of the bones is great the net absorption of dietary Ca via the intestinal mucosa can be much greater than normal. The main features of Ca balance include a miscible pool mainly in blood and extracellular fluids, which provides for the slow turnover in bones. The pool is replenished by dietary Ca and losses occur in urine.

It is very important to note that the concentration of plasma Ca is finely regulated and controls the size of the pool. Ca balance is hence actively controlled by a large number of factors. The external balance of Ca (the difference between intake and output) is, in effect, determined by the exchange between the skeleton, the intestine and the kidney. These fluxes are controlled by the action

of calciotropic hormones: parathyroid hormone; 1,25-dihydroxycholecalciferol; calcitonin. It is also influenced by other factors such as sex hormones, growth hormones, corticosteroids and a variety of locally-acting hormones (Fig. 4)⁽²⁰⁾.

Within bone, Ca is in the form of hydroxyapatite crystals (Ca₁₀(PO₄)₆(OH)₂), which also contain P and Mg, and contributes to its strength. P is found in abundance in a variety of foods but Mg is more limited and is an area of bone health nutrition that requires further attention.

Calcium and peak bone mass development

Peak bone mass is one of the key factors determining bone mass and fracture risk later in life. Attaining a high peak bone mass within an individual's genetic potential is a key preventative strategy for fracture reduction. Hence, the focus of research must be more fully directed on modifiable factors affecting the peak attainment of bone. It is known that genetic factors do play a key role in the variation of peak bone mass, with estimates of approximately 70–75%⁽²¹⁾, which still leaves plenty of room for key exogenous factors such as diet and physical activity to play a key role in bone mass development.

The length of time peak mass is maintained before age-related bone loss begins is approximately 28 years of age. There are some studies that indicate that the duration is relatively brief and others that indicate it may last several years. This disparity appears to depend on the skeletal region examined⁽²²⁾, and is likely to become clearer as more longitudinal studies are published. There are data, however, to show that both Ca absorption and bone Ca

deposition rates peak in girls shortly before menarche⁽²³⁾, which raises key questions as to the most effective timing of Ca and physical activity strategies for improving peak bone mass attainment.

Low Ca intakes during growth and late menarcheal age will affect peak bone mass, and consequently are likely to impact on fracture risk later in life. There are some studies that indicate that adolescent girls are less likely than boys to meet the current recommended dietary levels for Ca (although under-reporting may be a key confounding factor in these studies)⁽²⁴⁾.

Clinical trials with Ca supplements in both children and teenagers have shown an overall positive effect of Ca on bone mass accrual. The effect has been shown to be between 1 and 6% per year in the total body and between 1 and 10% per year at different specific skeletal regions when compared with a placebo⁽²⁵⁾. However, the studies have only been of short duration (between 1 and 3 years). Pubertal stage is critical in relation to the effectiveness of the supplement. For example, a study of adolescent girls post menarche (<15.5 years of age) who had baseline low Ca intakes (<800 mg/d) has shown enhanced bone mineral acquisition in those girls given a 1000 mg/d supplement in comparison with girls given a placebo⁽²⁶⁾. The effect was observed to be especially evident in girls who were >2 years past the onset of menarche.

In a follow-up study to a 3.5-year Ca supplementation trial in ninety-six adolescent girls multivariate analysis has revealed that total-body bone mineral density (BMD) accrual over the 7 years (original and follow-up study) in the active-treatment cohort is attributable to Ca supplementation⁽²⁷⁾. A further 8-year follow-up study of Ca supplementation in girls who were prepubertal has suggested that prepubertal Ca intake may also have an effect on the timing of menarche⁽²⁸⁾. The areal BMD gain between 7.9 and 16.4 years of age was found to be inversely related to menarcheal age at the axial and appendicular skeletal sites. A significant difference was observed between groups in relation to menarcheal age, with girls in the Ca-supplemented group starting menarche approximately 5 months sooner. On subgroup analysis based on menarcheal age it was found that the mean areal BMD gain from baseline in girls whose menarcheal age was below the median (13 years) remained significantly greater at all sites measured. In the late-menarche subgroup no lasting effect of Ca supplementation on BMD was observed. The authors suggest that Ca supplementation in prepubertal girls may accelerate the onset of pubertal maturation.

These data indicate the importance of Ca to peak bone mass development, although further research is clearly required to identify the exact mechanisms of an effect of increased Ca on pubertal timing⁽²⁹⁾.

Calcium and physical activity interactions: effects on peak bone mass attainment

Over 100 years ago, the German scientist Julius Wolff proposed the theory that is known as 'Wolff's Law': 'bone accommodates the forces applied to it by altering its amount and distribution of mass'⁽³⁰⁾. More recently, this

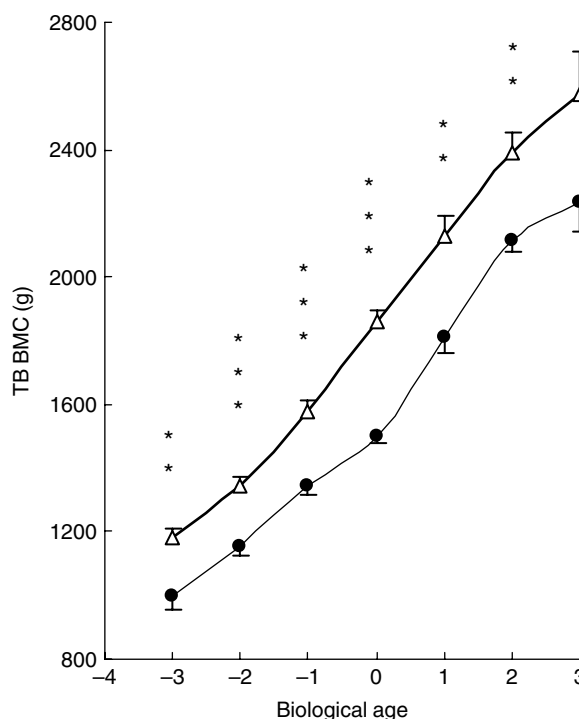


Fig. 5. Positive effect of impact loading exercise on peak bone mass attainment in young female gymnasts (Δ) in comparison with healthy controls (CON; \bullet) based on total-body (TB) bone mineral content (BMC) by maturity (biological age 0 being the age of puberty and peak height velocity). Values are means with their standard errors represented by vertical bars. When adjusted for height and weight mean values were significantly different from those for CON: ** $P < 0.01$, *** $P < 0.001$. (From Nurmi-Lawton *et al.*⁽³²⁾; reproduced with permission.)

concept has been refined to a general theory of bone mass regulation, known as the mechanostat model⁽³¹⁾. It is well known that in the absence of weight-bearing exercise bone loss will occur at both axial and appendicular skeletal sites. A 3-year longitudinal investigation has shown consistently higher bone mineral content in young girls regularly undertaking weight-bearing exercise in comparison with healthy controls, despite late age of menarche or amenorrhoea (Fig. 5)⁽³²⁾. Controlled intervention studies provide stronger evidence that effects of activity are causal and can be attained in less-active children who start to exercise (Fig. 6⁽³³⁾). Several such studies have examined the effects of exercise interventions on bone in children, with BMD or bone mineral content gains in the order of 1–5% greater in the exercise-intervention group than in the control groups^(33,34). Structural adaptation also seems to occur, including increases in bone area, bone circumference and bone width.

There are increasing data to suggest that both Ca and physical activity work synergistically on the skeleton⁽³⁵⁾. A randomised controlled trial in young children (3–5 years) has shown that exercise increases leg bone mineral content in children receiving a Ca supplement (1000 mg/d) but not in those receiving a placebo⁽³⁶⁾. Cortical thickness and area of the tibia have also been found to increase in response to activity in children taking Ca, but to decline in those

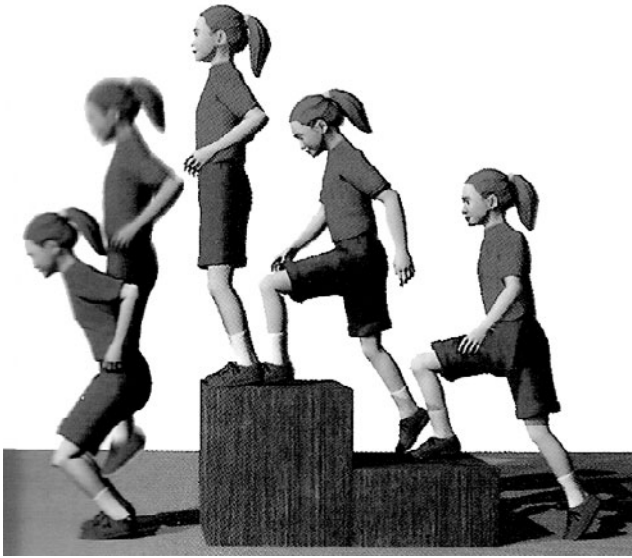


Fig. 6. An example of a jumping programme that improves hip and lumbar spine bone mass in prepubescent children. (From Fuchs *et al.*⁽³³⁾; reproduced with permission.)

receiving a placebo. Similar results have also been reported for older children (7–11 years); greater leg bone mineral accrual was found with a combination of a Ca supplement (800 mg/d) and exercise compared with either treatment alone⁽³⁷⁾. Hence, adequate Ca intake may be essential for optimal skeletal response to exercise and exercise might enhance effects of Ca at weight-bearing sites⁽³⁸⁾.

Calcium and post-menopausal bone loss

There are now good data to show that Ca supplements are effective in reducing bone loss in women late post menopause (>5 years post menopause), particularly in those with low habitual Ca intake (<400 mg/d)⁽³⁹⁾. A key study has shown that women late post menopause have a significant reduction in lumbar spine and femoral neck bone loss following supplementation with 500 mg Ca/d, with no such effect in women early post menopause (<5 years post menopause)⁽⁴⁰⁾ (Table 1). A review of over twenty studies has shown that Ca supplementation can decrease bone loss by approximately 1% per year⁽⁴¹⁾ and a meta-analysis that included fifteen trials indicates that Ca supplementation at levels between 500 and 2000 mg/d reduces post-menopausal bone loss⁽⁴²⁾; Ca changes were of the order of 1.66% at the lumbar spine and 1.64% at the hip. There are some data to suggest that the effect of Ca supplementation may be greater at skeletal sites with more cortical bone^(43,44). There are also data to suggest that Ca supplementation improves the efficacy of antiresorptive therapy on bone mass⁽⁴⁵⁾.

Vitamin D nutrition: key concepts

Types of vitamin D

Vitamin D is the generic term for two molecules, i.e. ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). Ergocalciferol is derived by UV irradiation of ergosterol,

Table 1. Calcium supplementation and post-menopausal bone loss in healthy women who received placebo or either calcium citrate malate (CCM) or calcium carbonate (CC; 500 mg calcium/d) for 2 years (from Dawson-Hughes *et al.*⁽⁴⁰⁾; reproduced with permission)

Treatment groups	Percentage change in lumbar spine BMD			
	After 1 year		After 2 years	
	Mean	SE	Mean	SE
CCM	0.60	0.56	0.41	0.69
CC	0.28	0.56	-0.07	0.69
Placebo	-0.72	0.49	-1.33	0.60

BMD, bone mineral density.

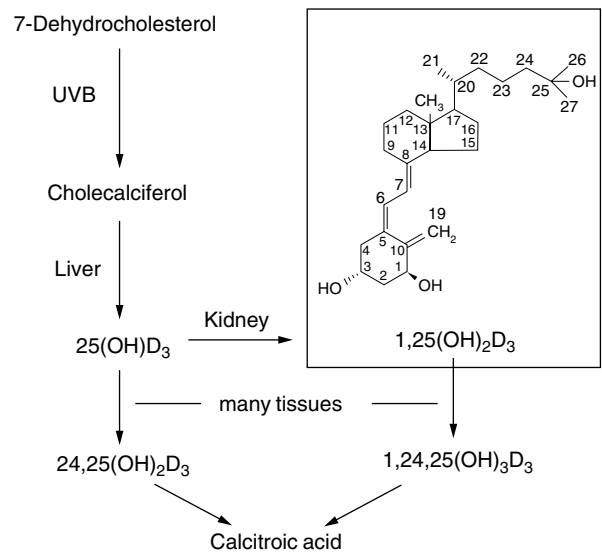


Fig. 7. Metabolism of vitamin D. 25(OH)D₃, 25-hydroxycholecalciferol; 1,25(OH)₂D₃, 1,25-dihydroxycholecalciferol; 24,25(OH)₂D₃, 24,25-dihydroxycholecalciferol; 1,24,25(OH)₃D₃, 1,24,25-trihydroxycholecalciferol. (From Holick⁽⁵⁴⁾; reproduced with permission.)

which is found in fungi and plants⁽⁴⁶⁾. Cholecalciferol is formed from the effect of UV irradiation on the skin and the efficiency of cholecalciferol synthesis is dependent on the number of UVB photons that penetrate into the epidermis. As shown in Fig. 7, the action of sunlight on the skin converts 7-dehydrocholesterol to previtamin D, which is metabolised to vitamin D by a temperature-dependent isomerisation. Vitamin D is then transported via the general circulation to the liver, where the enzyme 25-hydroxylase converts it to 25-hydroxycholecalciferol (25 OHD). The kidney is the site for further conversion to 1,25-dihydroxycholecalciferol. 25 OHD is the best indicator of clinical status and is the key circulating vitamin D metabolite⁽⁴⁷⁾. 1,25-Dihydroxycholecalciferol is the active form of the vitamin, which is involved in Ca homeostasis, helping to maintain normal blood levels of Ca and P and promoting Ca absorption and bone mineralisation⁽⁴⁸⁾.

Sources of vitamin D

It is believed that the main source of vitamin D is the UV in sunlight. However, whether UV exposure is sufficient



Fig. 8. Evidence of extensive hypovitaminosis D in the UK from the 1958 British Cohort (n 7437). 25-hydroxyvitamin D levels (nmol/l) of <25 (10 ng/ml), <40 (16 ng/ml) and <75 (30 ng/ml) were found in 15.5, 46.6 and 87.1% of the population respectively. Distribution in the population: (□), 5–9.9%; (▤), 10–19.9%; (▥), 20–29.9%; (▦), 30–39.9%; (▧), 40–49.9%; (▨), 50–59.9%; (■), 60–69.9%. (From Hyponnen & Powers⁽⁵³⁾; reproduced with permission.)

to keep vitamin D levels optimal has recently been the subject of considerable debate⁽⁴⁹⁾, particularly given the evidence of extensive hypovitaminosis D in the UK. Findings of the National Diet and Nutrition Surveys (4–18 years⁽⁵⁰⁾, 19–64 years⁽⁵¹⁾ and ≥ 65 years⁽⁵²⁾) indicate that vitamin D ‘deficiency’ (defined as a plasma 25 OHD level <25 nmol/l or 10 ng/ml) is a problem (e.g. 24% of men and 28% of women in the age-group 19–24 years have levels <25 nmol/l⁽⁵¹⁾). More recently, data from the 1958 British birth cohort (n 7437) has shown extensive hypovitaminosis D in subjects at 45 years of age; 25 OHD levels <25, <40 and <75 nmol/l were found in 15.5, 46.6 and 87.1% of the population respectively, with lower levels in the north *v.* south⁽⁵³⁾ (Fig. 8).

Much of the UV in sunlight is absorbed by clouds, O₃ and other forms of atmospheric pollution. With a reduced zenith angle of the sun and increased path length of sunlight through the atmosphere, the effective level of UV energy decreases north–south with distance from the seasonally-varying latitude at which the sun is directly overhead⁽⁵⁴⁾. Hence, in areas of northern latitude (such as the UK) there is no UV radiation of the appropriate wavelength (280 nm–310 nm) from the end of October to the end of March. For the remaining months of the year the main percentage of the effective UV radiation occurs between 11.00 hours and 15.00 hours⁽⁵⁵⁾.

A number of factors affect vitamin D production from UV exposure: (1) sunscreens absorb UVB radiation when applied to the skin; hence, there is marked reduction in the penetration of UVB photons into the epidermis and as a result production of pre-cholecalciferol is markedly reduced; (2) glass absorbs all UVB photons and hence exposure of the skin from sunlight that has passed through glass will not promote cholecalciferol synthesis in the skin; (3) clothing also absorbs UVB radiation and so no cholecalciferol is made in the skin covered by clothing; hence, women who cover up for cultural reasons are vitamin D

deficient and this factor represents an area of research that requires urgent attention.

As shown in Table 2, there are few dietary sources of vitamin D. The major providers are fat spreads (which are fortified with vitamin D), fish, eggs, pastry products, fortified breakfast cereals and meat⁽⁵⁶⁾. Recent changes in EU regulations have resulted in a number of cereal manufacturers removing the vitamin D fortification⁽⁵⁷⁾. Key Food Standards Agency-funded research on the interaction between diet and sunlight exposure on vitamin D status is currently underway and results will provide core information on vitamin D requirements⁽⁵⁸⁾.

Importance of vitamin D to bone

Vitamin D stimulates bone matrix formation and bone maturation. It also enhances osteoclastic activity and there are some data to suggest that it may influence differentiation of bone cell precursors⁽⁵⁹⁾. Together with parathyroid hormone, it regulates Ca and P metabolism and promotes Ca absorption from the gut and kidney tubules⁽⁶⁰⁾. It has been shown that fractional Ca absorption increases with serum 25 OHD concentrations within the reference range, up to a level of 80 nmol/l, reaching a plateau above that level⁽⁶¹⁾.

Vitamin D and peak bone mass development

There is growing evidence that mild vitamin D insufficiency can have a detrimental effect on bone mineral mass in adolescent females⁽⁶²⁾ and children^(63,64). A 3-year prospective cohort study in 171 healthy Finnish girls aged 9–15 years comparing subjects with severe hypovitaminosis D (25 OHD <20 nmol/l) with those with normal vitamin D status (25 OHD ≥ 37.5 nmol/l) has found a difference of 4% in BMD accrual⁽⁶⁴⁾. The adjusted change in

Table 2. Vitamin D content of foods* (from Lanham-New *et al.*⁽⁵⁶⁾; reproduced with permission)

Food	Vitamin D content (µg) per portion†
Cod liver oil (one tablespoon; 11 g)	23
Salmon steak, steamed (100 g)	8.7
Sardines, tinned in tomato sauce (100 g)	8.0
Tuna, tinned in brine (100 g)	3.6
Mackerel, one small, smoked (100 g)	8.0
Herring, one small fillet, grilled (85 g)	13.7
Pilchards, tinned in tomato sauce, small tin (50 g)	7.0
Minced beef, small portion, stewed (100 g)	0.8
Beef rump steak, grilled, (102 g)	0.4
Lamb, leg, roast (90 g)	0.6
Pork chop, grilled (120 g)	1.0
Liver (all types), stewed or fried (50 g)	0.15–1.1
Egg, one medium, boiled (61 g)	1.1
Fortified products	
Margarine, polyunsaturated, spread on two slices of bread (14 g)	1.1
Breakfast cereals fortified with vitamin D (30 g)†	0.6–2.5
Two types of fromage frais currently on the market fortified with vitamin D (100 g)†	0.9–1.3
A few types of yogurts currently on the market fortified with vitamin D (100 g)†	0.84–1.16
Two types of yogurt drinks currently on the market (90 g)†	0.7–1.1
Processed cheese 'triangle' fortified with vitamin D (approximately 22.5 g)†	1.0
Processed cheese slice fortified with vitamin D (approximately 25 g)†	0.9
Processed cheese strip fortified with vitamin D (approximately 21 g)†	0.9

*Portions are for adolescent boys and girls and adults and portions sizes (with the exception of items indicated) are from Food Standards Agency⁽⁹⁴⁾.

†Portion size and values taken from packaging information.

‡Values (with the exception of items indicated) are from McCance & Widdowson⁽⁹⁵⁾.

lumbar spine BMD was shown to be 27% higher for subjects in the highest tertile of vitamin D intake compared with those in the lowest tertile. Interestingly, the findings were reported to be significant only for girls at the post-pubertal stage, with no differences for girls of less maturity (<12 years); a finding that has been reported previously⁽⁶⁵⁾. Low vitamin D status is a risk factor for attaining a reduced peak bone mass⁽⁶⁶⁾.

In a key vitamin D-supplementation study on bone mineral accretion in 212 adolescent girls who were Ca replete (mean age 11.4 years) bone mineral augmentation at the femur was found to be 14.3 and 17.2% higher in the groups receiving the vitamin D supplementation (at either 5 µg/d or 10 µg/d) in comparison with the placebo group⁽⁶⁷⁾. The findings followed a dose–response relationship for those subjects who were Ca replete. A difference was also seen for lumbar spine bone density between the group supplemented at 10 µg/d level and the placebo group. Furthermore, vitamin D supplementation was found to significantly reduce bone resorption (as assessed by urinary deoxypyridinoline excretion). These findings were

only observed to be significant when the data were analysed by the compliance-based method (which includes only participants who complied with instructions) as opposed to the intention-to-treat method (which includes all participants who entered the trial). Further research is required on the combined effect of Ca and vitamin D on maximising peak bone mass attainment, particularly in children accustomed to a low Ca intake.

Calcium and vitamin D in fracture prevention

Ca and vitamin D supplementation studies have been shown to reduce fracture rates in institutionalised elderly individuals⁽⁶⁸⁾. Women living in nursing homes in France were given a 1200 mg Ca supplement and 20 µg vitamin D daily for a period of 18 months. Both hip fracture ($P=0.040$) and non-vertebral fractures ($P=0.015$) were shown to be significantly reduced in those women on the active treatment in comparison with the placebo (Fig. 9).

There are also data to suggest an effect of Ca and vitamin D on fracture prevention in free-living elderly populations. Elderly American men and women (mean age 71 years) given 500 mg Ca and 20 µg cholecalciferol daily were found to have a reduced total number of non-vertebral fractures⁽⁶⁹⁾. In a factorial cluster-randomised pragmatic intervention study of 9605 community-dwelling residents living in a northern European region (Denmark) a daily supplement of CaCO₃ (1000 mg) and vitamin D (10 µg) over a period of 3 years was reported to result in a 16% reduction in fracture incidence rate (relative risk 0.84 (95% CI 0.72, 0.98) in treated subjects compared with those subjects who were offered no supplement but participated in an environmental and health programme⁽⁷⁰⁾.

Vitamin D supplementation alone is not effective in preventing fractures in the elderly if the dosage is limited. For example, a supplementation trial undertaken in Norway using cod liver oil that provided 10 µg vitamin D/d was not found to prevent osteoporotic fractures in 1144 nursing home residents⁽⁷¹⁾. It is important to note that a recent meta-analysis indicates that 17.5–20 µg/d reduces the risk of hip and any non-vertebral fractures in ambulatory or institutionalised elderly individuals, but 10 µg/d is not effective⁽⁷²⁾.

A recently published study examining the effect of three injections of 7500 µg cholecalciferol per year has found no reduction in fracture risk in 9000 healthy ambulatory elderly men and women and, if anything, has shown that the vitamin D supplementation increased the risk of fracture ($P<0.06$)⁽⁷³⁾. This finding contrasts with the results of a study of elderly British men and women aged 65–85 years in which it was shown that supplementation with 2500 µg cholecalciferol orally every fourth month for 5 years reduces the number of fractures of the hip, wrist, forearm and spine by 22% compared with placebo⁽⁷⁴⁾.

Two key UK studies have not demonstrated a positive effect of Ca and vitamin D supplementation on fracture prevention in the free-living elderly population^(75,76). In a pragmatic open randomised trial conducted with 3314 women aged ≥70 years who had risk factors for hip fracture Ca (1000 mg/d) and cholecalciferol (20 µg/d)

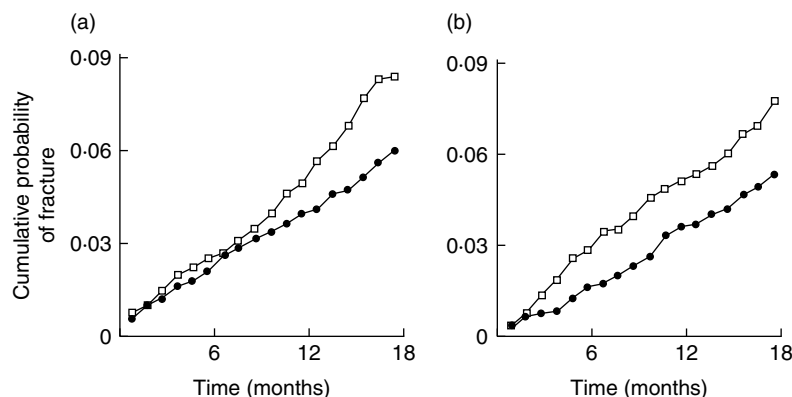


Fig. 9. Cumulative probability of hip fracture (a) and other non-vertebral fractures (b) in a placebo group (□) compared with a group treated with a calcium (1.2 g/d; ●) and vitamin D (20 µg/d) supplement. Hip fracture ($P = 0.040$) and non-vertebral fractures ($P = 0.015$) were significantly reduced for the supplemented group compared with the placebo. (From Chapuy *et al.*⁽⁶⁸⁾; reproduced with permission.)

supplementation was not found to reduce the risk of fracture⁽⁷⁵⁾. No evidence of an effect on falls at either 6 months (OR 0.99 (95% CI 0.81, 1.20)) or 1 year (OR 0.98 (95% CI 0.79, 1.20)) were seen. Adherence rate (60%), however, was low. In the MRC-Record trial involving 5292 men and women aged ≥ 70 years who were mobile before developing a low-trauma fracture, 1000 mg Ca and 20 µg vitamin D, either alone or in combination, was not found to significantly reduce the incidence of new low-trauma fractures after 2 years⁽⁷⁶⁾. It is important to note, however, that compliance was again low in this study (possibly as low as 45% when non-responders to the questionnaire were included) and only a small number of subjects were measured for vitamin D status⁽⁷⁷⁾.

The 2005 Cochrane review has reported a reduced risk for hip fracture (relative risk 0.81 (95% CI 0.68, 0.96) for seven trials) and non-vertebral fractures (relative risk 0.87 (95% CI 0.78, 0.97) for seven trials) but no significant effect on vertebral fractures⁽⁷⁸⁾. The authors conclude that the effect may be restricted to those living in institutional care. The most recent meta-analysis of the effect of Ca and vitamin D supplements on fracture prevention suggests an overall positive effect⁽⁷⁹⁾.

Vitamin K nutrition and bone

Vitamin K ('koagulation vitamin') was first described as a dietary-derived coagulation factor⁽⁸⁰⁾; it was noted that a bleeding disorder in chickens was corrected by feeding a variety of vitamin K-rich diets. The fat-soluble vitamin was finally isolated in 1939⁽⁸¹⁾. Vitamin K refers to a family of compounds with a common chemical structure, 2-methyl-1,4 naphthoquinone, as shown in Fig. 10. Phylloquinone (vitamin K₁) is present in foods of plant origin. Bacterial forms of vitamin K, referred to as the menaquinones (vitamin K₂) differ in structure from phylloquinone⁽⁸²⁾.

Vitamin K has an important function for the skeleton as it acts as a cofactor in the post-translational carboxylation of several bone proteins, with osteocalcin being the most

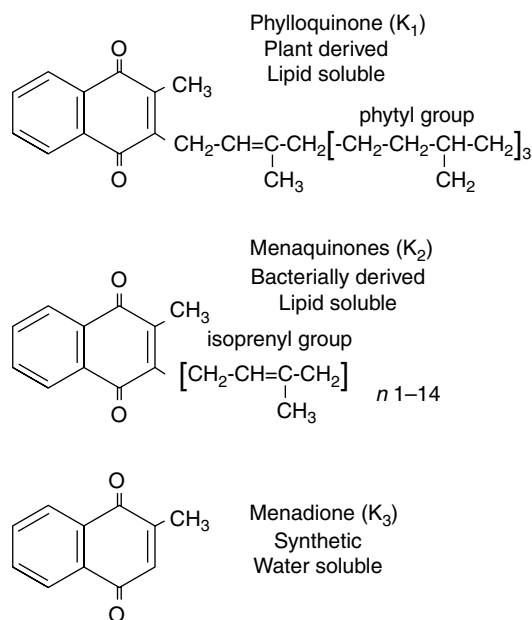


Fig. 10. Different forms of vitamin K. Vitamin K exists in two forms, phylloquinone and menaquinones.

abundant⁽⁸³⁾. Deficiency of vitamin K results in the synthesis of under-carboxylated osteocalcin⁽⁸⁴⁾. There are data to show that low serum concentrations of either phylloquinone or under-carboxylated osteocalcin are associated with low BMD and increased risk for osteoporotic fracture⁽⁸⁵⁾.

A number of recently published studies suggest a key role for vitamin K nutrition for optimisation of bone health⁽⁸⁶⁾. Phylloquinone supplementation (at a dose of 200 µg/d, which would be obtainable in the diet from a 50 g portion of green leafy vegetables) in combination with Ca (1000 mg) and vitamin D (10 µg) was investigated over a 2-year period in a randomised placebo-controlled study⁽⁸⁷⁾. Subjects who took the combined phylloquinone and

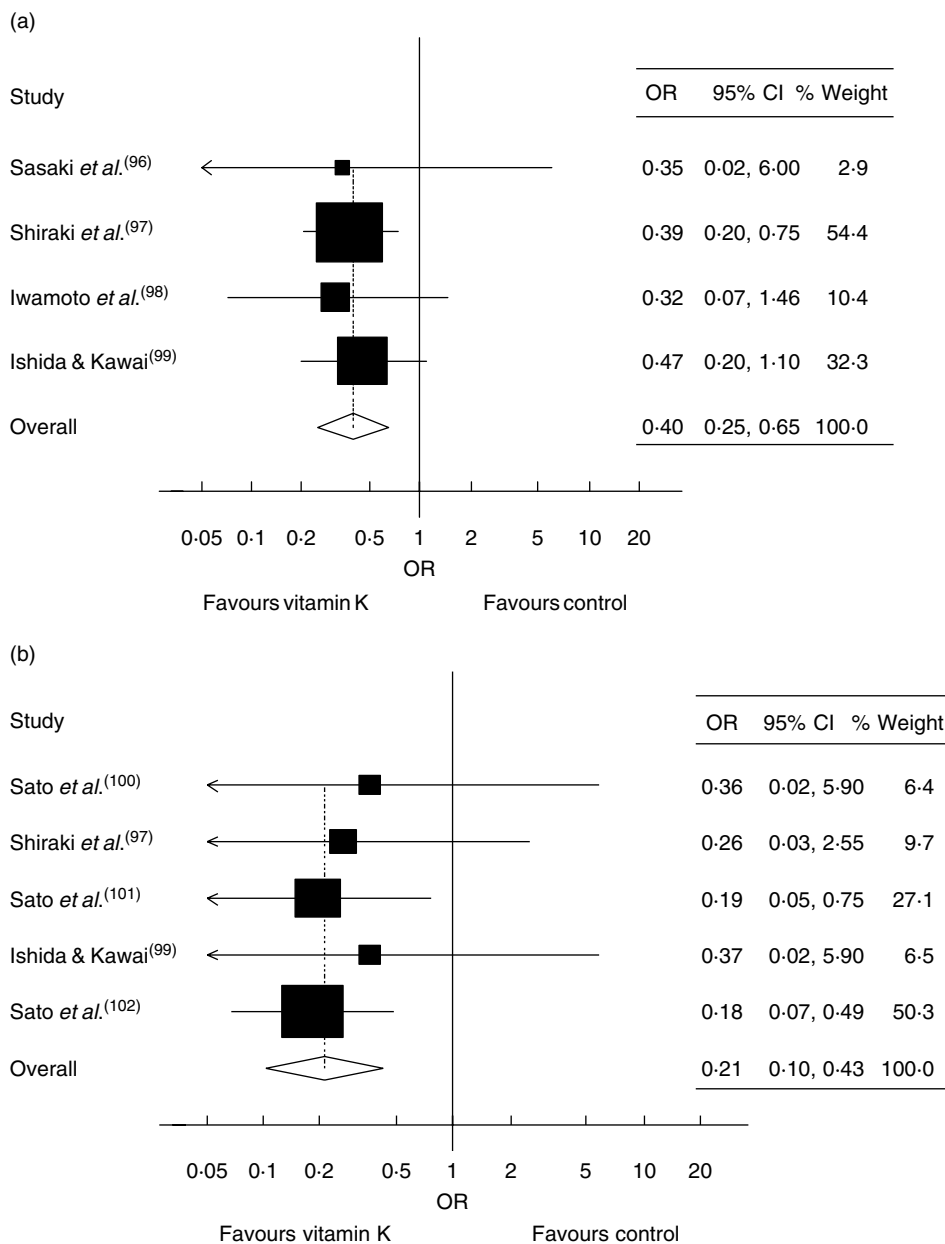


Fig. 11. Meta-analysis of the effect of menaquinones on (a) vertebral fracture prevention and (b) hip fracture prevention. (From Cockayne *et al.*⁽⁹⁰⁾; reproduced with permission.)

vitamin D plus Ca supplement were found to show a significant increase in ultra-distal BMD and bone mineral content. The effect of phylloquinone (at a higher dose of 1 mg/d) on BMD has been studied in 155 healthy postmenopausal women aged 50–60 years of age over 36 months⁽⁸⁸⁾. The study examined the potential complementary effect on post-menopausal bone loss of phylloquinone, vitamin D (8 µg/d) and a mineral supplement (including 150 mg Mg/d, 500 mg Ca/d and 10 mg Zn/d). The results indicate a positive effect of the phylloquinone supplement on bone loss at the femoral neck, leading to a 35% reduction in bone loss when compared with placebo after a period of 36 months.

Menaquinone supplementation has also been shown to effectively improve markers of bone health, although it is

important to note that the doses of menaquinones used could not be achieved via the diet⁽⁸⁹⁾. A recent meta-analysis examining the effect of menaquinone supplementation on vertebral and hip fracture shows a convincing effect of pharmacological doses of menaquinones on fracture rate (Fig. 11(a and b))^(90,91). The role of vitamin K on prevention (and possibly treatment) of osteoporosis is an exciting area that requires further research.

Concluding remarks

It is widely recognised that there are genetic, environmental, lifestyle and dietary determinants of risk of osteoporotic fracture, as well as interactions between them.

There is good evidence to show that the effects of nutrition on the skeleton are powerful and wide-ranging. Given that by the year 2030 one in four of the adult population will be elderly, it is vital that special attention is given to nutritional strategies for the optimisation of bone health throughout the life cycle, including the role of diet in reducing falls⁽⁹²⁾. Ca and vitamin D are clearly key nutrients for optimal bone health. At all costs, suboptimal intakes or status must be prevented and dietary vitamin D fortification should be considered for vulnerable groups such as older adults, post-menopausal women, adolescent females and amenorrhoeic women. Ca and vitamin D have been shown to be effective strategies for fracture prevention in the elderly, particularly for those populations in which vitamin D insufficiency is rife. Ca supplementation alone does not appear to be effective in reducing fracture⁽⁹³⁾. In recent years, evidence has emerged for a role for vitamin K in bone health. Further data are urgently required to enable a fuller understanding of the complex interaction between dietary factors and bone health.

Acknowledgement

The author has the following conflicts of interest to declare: S.L-N. is co-director of D3TEX Ltd, a company which has a patent pending on materials which allow sunlight through. S.L-N. has also acted as a consultant to the following companies with respect to Vitamin D and Vitamin K: Dairy Crest Yoplait, CH Hansen, Springfields Ltd. The views expressed are the author's alone.

References

1. Parfitt AM (1990) Osteomalacia and related disorders. In *Metabolic Bone Disease and Clinically Related Disorders*, 2nd ed., pp. 329–396 [LV Avioli and SM Krane, editors]. Philadelphia, PA: WB Saunders.
2. Loveridge N & Lanham-New SA (2008) Bones and ageing. In *British Nutrition Foundation Taskforce on Nutritional Aspects of Ageing* [J Mathers and J Buttriss, editors]. London: Blackwell Publishing (In the Press).
3. Eastell R (1999) Pathogenesis of postmenopausal osteoporosis. In *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 4th ed., pp. 260–262 [MJ Favus, editor]. London: Williams & Wilkins.
4. Consensus Development Conference (1991) Diagnosis, prophylaxis and treatment of osteoporosis. *Am J Med* **90**, 107–110.
5. Consensus Development Conference (1993) Diagnosis, prophylaxis and treatment of osteoporosis. *Am J Med* **94**, 646–650.
6. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V & Weaver C (2000) Peak bone mass. *Osteoporos Int* **11**, 985–1009.
7. van Staa TP, Dennison EM, Leufkens HG & Cooper C (2001) Epidemiology of fractures in England and Wales. *Bone* **29**, 517–522.
8. Dennison E & Cooper C (2007) Lifestyle and constitutional risk factors. In *Managing Osteoporosis*, pp. 69–80 [SA Lanham-New, T O'Neill, R Morris, D Skeleton and A Sutcliffe, editors]. Oxford: Clinical Publishing.
9. Torgerson DJ, Iglesias C & Reid DM (2001) *Economics of Osteoporosis. Key Advance Series*. London: Aesculapius Press.
10. World Health Organization (1994) *Study Group on Assessment of Fracture Risk and Its Application to Screening and Postmenopausal Osteoporosis. Report of a WHO Study Group. Technical Report Series no. 84*. Geneva: WHO.
11. Abrams SA (2003) Normal acquisition and loss of bone mass. *Horm Res* **60**, 71–76.
12. Smith R (2003) Calcium and the bone minerals. In *Human Nutrition and Dietetics*, pp. 451–489 [WPT James and J Garrow, editors]. Cambridge: Cambridge University Press.
13. National Institutes of Health Consensus Development Panel (2000) Osteoporosis Prevention, Diagnosis, and Therapy. NIH Consens Statement Online 2000 March 27–29 17(1), 1–36. <http://consensus.nih.gov/2000/2000Osteoporosis111html.htm> (accessed January 2006).
14. The National Osteoporosis Society (2006) What is osteoporosis? <http://www.nos.org.uk/osteo.asp> (accessed January 2006).
15. Royal College of Physicians (2000) *Osteoporosis Clinical Guidelines for Prevention and Treatment*. London: Royal College of Physicians of London.
16. Department of Health (1998) *Nutrition and Bone Health: With Particular References to Calcium and Vitamin D. Report on Health and Social Subjects 49*. London. H. M. Stationery Office.
17. Food and Nutrition Board of the Institute of Medicine (1997) *Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin, and Fluoride*. Washington, DC: National Academy Press.
18. Prentice A, Schoenmakers I, Laskey MA, de Bono S, Ginty F & Goldberg GR (2006) Nutrition and bone growth and development. *Proc Nutr Soc* **65**, 348–360.
19. Abrams SA (2005) Calcium supplementation during childhood: long-term effects on bone mineralization. *Nutr Rev* **63**, 251–255.
20. Ward KA, Roberts SA, Adams JE, Lanham-New SA & Mughal MZ (2007) Calcium supplementation and weight bearing physical activity – do they have a combined effect on the bone density of pre-pubertal children? *Bone* **41**, 496–504.
21. Ralston S (2007) Genetics of osteoporosis. In *Managing Osteoporosis*, pp. 81–94 [SA Lanham-New, T O'Neill, R Morris, D Skeleton and A Sutcliffe, editors]. Oxford: Clinical Publishing.
22. Seeman E & Hopper J (1997) Genetic and environmental components of the population variance in bone density. *Osteoporos Int* **7**, S10–S16.
23. Langman CB (2005) Genetic regulation of bone mass: from bone density to bone strength. *Pediatr Nephrol* **20**, 352–355; Epublication 5 January 2005.
24. Lorentzon M, Mellstrom D & Ohlsson C (2005) Age of attainment of peak bone mass is site specific in Swedish men – The GOOD study. *J Bone Miner Res* **20**, 1223–1227; Epublication 7 March 2005.
25. Nieves J (2007) Calcium and vitamin D. In *Managing Osteoporosis*, pp. 21–33 [SA Lanham-New, T O'Neill, R Morris, D Skeleton and A Sutcliffe, editors]. Oxford: Clinical Publishing.
26. Rozen GS, Rennert G, Dodiuk-Gad RP, Rennert HS, Ish-Shalom N, Diab G, Raz B & Ish-Shalom S (2003) Calcium supplementation provides an extended window of opportunity for bone mass accretion after menarche. *Am J Clin Nutr* **78**, 993–998.
27. Bonjour JP, Ammann P, Chevalley T, Ferrari S & Rizzoli R (2003) Nutritional aspects of bone growth: an overview. In *Nutritional Aspects of Bone Health*, pp. 111–128

- [SA New and JP Bonjour, editors]. Cambridge: Royal Society of Chemistry.
28. Chevalley T, Rizzoli R, Hans D, Ferrari S & Bonjour JP (2005) Interaction between calcium intake and menarcheal age on bone mass gain: an eight-year follow-up study from prepuberty to postmenarche. *J Clin Endocrinol Metab* **90**, 44–51; Epublication 26 October 2004.
 29. Abrams SA, Copeland KC, Gunn SK, Gundberg CM, Klein KO & Ellis KJ (2000) Calcium absorption, bone mass accumulation and kinetics increase during early pubertal development in girls. *J Clin Endocrinol Metab* **85**, 1805–1809.
 30. Wolff J (1892) *Das Gesetz der Transformation der Knochen (The Law of Bone Remodelling)*. Berlin: Verlag von August Hirschwald.
 31. Frost HM (1987) Bone 'mass' and the 'mechanostat': a proposal. *Anat Rec* **219**, 1–9.
 32. Nurmi-Lawton JA, Baxter-Jones ADJ, Mirwald R, Bishop JA, Taylor P, Cooper C & New SA (2004) Evidence of sustained skeletal benefits from impact-loading exercise in young adolescent females: a 3-year longitudinal study. *J Bone Miner Res* **19**, 314–322.
 33. Fuchs RK, Bauer JJ & Snow CM (2001) Jumping improves hip and lumbar spine bone mass in prepubescent children: A randomized controlled trial. *J Bone Miner Res* **16**, 148–156.
 34. Valdimarsson O, Linden C, Johnell O, Gardsell P & Karlsson M (2006) Daily physical education in the school curriculum in prepubertal girls during 1 year is followed by an increase in bone mineral accrual and bone width – Data from the Prospective Controlled Malmo Pediatric Osteoporosis Prevention Study. *Calcif Tissue Int* **78**, 65–71.
 35. Specker BL (1996) Evidence for an interaction between calcium intake and physical activity on changes in bone mineral density. *J Bone Miner Res* **11**, 1539–1544.
 36. Specker B & Binkley T (2003) Randomized trial of physical activity and calcium supplementation on bone mineral content in 3- to 5-year-old children. *J Bone Miner Res* **18**, 885–892.
 37. Bass SL, Naughton G, Saxon L, Iuliano-Burns S, Daly R, Briganti EM, Hume C & Nowson C (2007) Exercise and calcium combined results in a greater osteogenic effect than either factor alone: A blinded randomized placebo-controlled trial in boys. *J Bone Miner Res* **22**, 458–464.
 38. Dawson-Hughes B (2004) Calcium and vitamin D for bone health in adults. In *Nutrition and Bone Health*, pp. 197–210 [MF Holick and B Dawson-Hughes, editors]. Totowa, NJ: Humana Press.
 39. Heaney RP (2000) There should be a dietary guideline for calcium. *Am J Clin Nutr* **71**, 658–670.
 40. Dawson-Hughes B, Dallal GE, Krall EA, Sadowski L, Sahyoun N & Tannenbaum S (1990) A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. *N Engl J Med* **323**, 878–883.
 41. Goulding A & Grant A (2007) Nutritional strategies to optimize bone health throughout the life course. In *Managing Osteoporosis*, pp. 3–20 [SA Lanham-New, T O'Neill, R Morris, D Skeleton and A Sutcliffe, editors]. Oxford: Clinical Publishing.
 42. Shea B, Wells G, Cranney A *et al.* (2002) Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group. Meta-analysis of therapies for post-menopausal osteoporosis. VII Meta-analysis of Ca supplementation for the prevention of postmenopausal osteoporosis. *Endocr Rev* **23**, 552–559.
 43. Ho SC, Chen YM, Woo JL & Lam SS (2004) High habitual calcium intake attenuates bone loss in early post-menopausal Chinese women: an 18-month follow up study. *J Clin Endocrinol* **89**, 2166–2170.
 44. Suzuki Y, Davison KS & Chilibeck PD (2003) Total calcium intake is associated with cortical bone mineral density in a cohort of postmenopausal women not taking oestrogen. *J Nutr Health Aging* **7**, 296–299.
 45. Nieves JW, Komar L, Cosman F & Lindsay R (1998) Calcium potentiates the effect of oestrogen and calcitonin on bone mass: review and analysis. *Am J Clin Nutr* **67**, 18–24.
 46. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266–281.
 47. Scientific Advisory Committee on Nutrition (2007) *Update on Vitamin D. Position Statement by the Scientific Advisory Committee on Nutrition*. London: The Stationery Office; available at http://www.sacn.gov.uk/pdfs/sacn_position_vitamin_d_2007_05_07.pdf
 48. Calvo MS, Whiting SJ & Barton CN (2005) Vitamin D intake: a global perspective of current status. *J Nutr* **135**, 310–316.
 49. Veith R, Bischoff-Ferrari H, Boucher BJ *et al.* (2007) The urgent needs to recommend an intake of vitamin D that is effective. *Am J Clin Nutr* **85**, 649–650.
 50. Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC (1998) *National Diet and Nutrition Survey of People Aged 65 Years and Over*. London: H. M. Stationery Office.
 51. Gregory J, Lowe S, Bates CJ, Prentice A, Jackson LV, Smithers G, Wenlock R & Farron M (2000) *National Diet and Nutrition Survey of People Aged 4–18 Years*. vol. 1. *Report of the Diet and Nutrition Survey*. London: The Stationery Office.
 52. Henderson L, Gregory J & Swan G (2002) *National Diet and Nutrition Survey: Adults Aged 19–64 Years*. vol. 1: *Types and Quantities of Foods Consumed*. London: The Stationery Office.
 53. Hypponen E & Power C (2007) Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr* **85**, 860–888.
 54. Holick MF (1994) Vitamin D: new horizons for the 21st century. *Am J Clin Nutr* **60**, 619–630.
 55. Department of Health (1991) *Dietary Reference Values for Food, Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects* no. 41. London: H. M. Stationery Office.
 56. Lanham-New SA, Thompson RL, More J, Brooke-Wavell K, Hunking P & Medici E (2007) Importance of vitamin D, calcium and exercise to bone health with specific reference to children and adolescents. *Nutr Bull* **32**, 364–377.
 57. Cashman K (2007) Other key nutritional factors. In *Managing Osteoporosis*, pp. 49–65 [SA Lanham-New, T O'Neill, R Morris, D Skeleton and A Sutcliffe, editors]. Oxford: Clinical Publishing.
 58. Ashwell M, Stone E, Mathers J *et al.* (2008) Review of the nutrition and bone health projects funded by the Food Standards Agency – have they helped to inform public health policy? *Br J Nutr* (In the Press).
 59. Underwood JL & DeLuca HF (1984) Vitamin D is not directly necessary for bone growth and bone mineralization. *Am J Physiol Endocrinol Metab* **246**, E492–E498.
 60. Boland R (1986) Role of vitamin D in skeletal muscle function. *Endocr Rev* **7**, 434–447.
 61. Heaney RP, Dowell MS & Hale CA (2003) Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J Am Coll Nutr* **22**, 142–146.
 62. Outila TA, Kakkainen MU & Lamberg-Allardt CJ (2001) Vitamin D status affects serum parathyroid hormone

- concentrations during winter in female adolescents: associations with forearm bone mineral density. *Am J Clin Nutr* **74**, 206–210.
63. Lehtonen-Veromaa MK, Mottonen TT, Nuotio IO, Irjala KMA, Leino AE & Viikari JSA (2002) Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. *Am J Clin Nutr* **76**, 1446–1453.
 64. Cheng S, Tylavsky F, Kroger H *et al.* (2003) Association of low 25-hydroxvitamin D concentrations with elevated parathyroid hormone concentrations and low cortical bone density in early pubertal and prepubertal Finnish girls. *Am J Clin Nutr* **78**, 485–492.
 65. El-Hajj Fuleihan G & Veith R (2007) Vitamin D insufficiency and musculoskeletal health in children and adolescents. In *Nutritional Aspects of Osteoporosis 2006*, pp. 91–108 [B Burckhard, RP Heaney and B Dawson-Hughes, editors]. Oxford: Elsevier.
 66. El-Hajj Fuleihan G, Nabulsi M, Choucair M, Salamoun M, Hajj Shahine C, Kizirian A & Tannous R (2001) Hypovitaminosis D in healthy schoolchildren. *Pediatrics* **107**, E53.
 67. Viljakainen HT, Natri AM, Karkkainen M, Huttunen MM, Palssa A, Jakobsen J, Cashman KD, Molgaard C & Lamberg-Allardt C (2006) A positive dose-response effect of vitamin D supplementation on site-specific bone mineral augmentation in adolescent girls: a double-blinded randomized placebo-controlled 1-year intervention. *J Bone Miner Res* **21**, 836–844.
 68. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD & Meunier PJ (1992) Vitamin D and calcium to prevent hip fractures in elderly women. *N Engl J Med* **327**, 1637–1642.
 69. Dawson-Hughes B, Harris SS, Krall EA & Dallal GE (1997) Effect of Ca and vitamin D supplementation on bone density in men and women 65 years of age or older. *N Engl J Med* **337**, 670–676.
 70. Larsen ER, Mosekilde L & Foldspang A (2004) Vitamin D and calcium supplementation prevents osteoporotic fractures in elderly community dwelling residents: a pragmatic population-based 3-year intervention study. *J Bone Miner Res* **19**, 370–378.
 71. Meyer HE, Smedshaug GB, Kvaavik E, Falch JA, Tverdal A & Pedersen JI (2002) Can vitamin D supplementation reduce the risk of fracture in the elderly? A randomized controlled trial. *J Bone Miner Res* **17**, 709–715.
 72. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T & Dawson-Hughes B (2005) Fracture prevention with vitamin D supplementation: a meta-analysis of randomised controlled trials. *JAMA* **293**, 2257–2264.
 73. Smith H, Anderson F, Raphael H, Maslin P, Crozier S & Cooper C (2007) Effect of annual intramuscular vitamin D on fracture risk in elderly men and women – a population-based, randomized, double-blind, placebo-controlled trial. *Rheumatology* **46**, 1852–1857.
 74. Trivedi DP, Doll R & Khaw KT (2003) Effect of four monthly oral vitamin D3 supplementation on fractures and mortality in men and women living in the community: a randomised double blind controlled trial. *Br Med J* **326**, 469–474.
 75. Porthouse J, Cockrayne S, King C *et al.* (2005) Randomised controlled trial of calcium supplementation with cholecalciferol (vitamin D3) for prevention of fractures in primary care. *Br Med J* **330**, 1003–1006.
 76. Grant AM, Avenell A, Campbell MK *et al.* (2005) Oral vitamin D3 and calcium for the secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. *Lancet* **365**, 1621–1628.
 77. Sambrook P (2005) Vitamin D and fractures: quo vadis? *Lancet* **365**, 1599–1600.
 78. Avenell A, Gillespie W, Gillespie L & O'Connell D (2005) Vitamin D and vitamin D analogues for preventing fractures associated with involutional and postmenopausal osteoporosis. *The Cochrane Database of Systematic Reviews* 2005, issue 3, CD000227. <http://www.cochrane.org/reviews/en/ab000227.html>
 79. Tang BM, Eslick GD, Nowson C, Smith C & Bensoussan A (2007) Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet* **370**, 657–666.
 80. Dam H (1935) The antihemorrhagic factor of the chick. *Biochem J* **29**, 1273–1285.
 81. Bolton-Smith C (1993) Vitamin K. In *Human Nutrition and Dietetics*, pp. 208–238 [WPT James and JS Garrow, editors]. Edinburgh: Churchill Livingstone.
 82. Shearer MJ, Bach A & Kohlmeier M (1996) Chemistry, nutritional sources, tissue distribution and metabolism of vitamin K with special reference to bone health. *J Nutr* **126**, 1181S–1186S.
 83. Booth S (2003) Dietary vitamin K and bone health. In *Nutritional Aspects of Bone Health*, pp. 323–338 [SA New and JP Bonjour, editors]. Cambridge: Royal Society of Chemistry.
 84. Szulc P, Chapuy M-C, Meunier PJ & Delmas PD (1996) Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture: a three year follow up study. *Bone* **18**, 487–488.
 85. Binkley NC, Krueger DC, Engelke JA, Foley AL & Suttie JW (2000) Vitamin K supplementation reduces serum concentrations of under-gamma carboxylated osteocalcin in healthy young and elderly adults. *Am J Clin Nutr* **72**, 1523–1528.
 86. Weber P (2001) Vitamin K and bone health. *Nutrition* **17**, 880–887.
 87. Bolton-Smith C, McMurdo ME, Paterson CR, Mole PA, Harvey JM, Fenton ST, Prynne CJ, Mishra GD & Shearer MJ (2007). A two-year randomized controlled trial of vitamin K1 (phylloquinone) and vitamin D3 plus calcium on the bone health of older women. *J Bone Miner Res* **22**, 509–519.
 88. Braam LA, Knapen MH, Geusens P, Brouns F, Hamulyák K, Gerichhausen MJ & Vermeer C (2003) Vitamin K1 supplementation retards bone loss in postmenopausal women between 50 and 60 years of age. *Calcif Tissue Int* **73**, 21–26.
 89. Knapen MHJ, Schurgers LJ & Vermeer C (2007) Vitamin K2 improves hip bone geometry and bone strength indices in postmenopausal women. *Osteoporos Int* **18**, 963–972; Epublication 8 February 2007.
 90. Cockrayne R, Adamson J, Lanham-New SA, Shearer MJ, Gilbody S & Torgerson DJ (2006) Vitamin K and the prevention of fractures: systematic review and meta-analysis of randomized controlled trials. *Arch Intern Med* **166**, 1256–1261.
 91. Shearer MJ, Cockrayne R, Adamson J, Lanham-New SA, Gilbody S & Torgerson DJ (2007) Vitamin K and bone health. *Arch Intern Med* **167**, 94–95.
 92. Jarvinen TLN, Sievanen H, Khan KM, Heinonen A & Kannus P (2008) Shifting the focus in fracture prevention from osteoporosis to falls. *Br Med J* **336**, 124–126.
 93. Bischoff-Ferrari HA, Dawson-Hughes B, Baron JA *et al.* (2007) Calcium intake and hip fracture risk in men

- and women: a meta-analysis of prospective cohort studies and randomized controlled trials. *Am J Clin Nutr* **86**, 1780–1790.
94. Food Standards Agency (2002) *Food Portion Sizes*, 3rd ed. London: The Stationery Office.
95. McCance RA & Widdowson EM (2004) *The Composition of Foods*, 6th ed. Cambridge: Royal Society of Chemistry.
96. Sasaki N, Kusano E, Takahashi H, Ando Y, Yano K, Tsuda E & Asano Y (2005) Vitamin K2 inhibits glucocorticoid-induced bone loss partly by preventing the reduction of osteoprotegerin (OPG). *J Bone Miner Metab* **23**, 41–47.
97. Shiraki M, Shiraki Y, Aoki C & Miura M (2000) Vitamin K2 (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. *J Bone Miner Res* **15**, 515–522.
98. Iwamoto J, Takeda T & Ichimura S (2001) Effect of menatetrenone on bone mineral density and incidence of vertebral fractures in postmenopausal women with osteoporosis: a comparison of with the effect of etidronate. *J Orthop Sci* **6**, 487–492.
99. Ishida Y & Kawai S (2004) Comparative efficacy of hormone replacement therapy, etidronate, calcitonin, alfacalcidol and vitamin K in postmenopausal women with osteoporosis: The Yamaguchi Osteoporosis Prevention Study. *Am J Med* **117**, 549–555.
100. Sato Y, Honda Y, Kuno H & Oizumi K (1998) Menatetrenone ameliorates osteopenia in disuse-affected limbs of vitamin D and K-deficient stroke patients. *Bone* **23**, 291–296.
101. Sato Y, Honda Y, Asho T, Hosokawa K, Kondo I & Satoh K (2002) Amelioration of osteoporosis by menatetrenone in elderly female Parkinson's disease patients with vitamin D deficiency. *Bone* **31**, 114–118.
102. Sato Y, Kanoko T, Satoh K & Iwanamoto J (2005) Menatetrenone and vitamin D2 with calcium supplements prevent nonvertebral fractures in elderly women with Alzheimer's disease. *Bone* **36**, 61–68.