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Review Article

Minerals and vitamins in bone health: the potential value of dietary enhancement

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Nutrition is important to bone health, and a number of minerals and vitamins have been identified as playing a potential role in the prevention of bone diseases, particularly osteoporosis. Despite this, there is currently no consensus on maximum levels to allow in food or as dietary supplements. The benefits of supplementation of populations at risk of osteoporosis with Ca and vitamin D are well established. Prolonged supplementation of Ca and vitamin D in elderly has been shown to prevent bone loss, and in some intervention studies to prevent fragility fractures. Although P is essential to bone health, the average intake is considered to be more than sufficient and supplementation could raise intake to adverse levels. The role of vitamin K in bone health is less well defined, though it may enhance the actions of Ca and vitamin D. Sr administered in pharmacological doses as the ranelate salt was shown to prevent fragility fractures in postmenopausal osteoporosis. However, there is no hard evidence that supplementation with Sr salts would be beneficial in the general population. Mg is a nutrient implicated in bone quality, but the benefit of supplementation via foodstuffs remains to be established. A consensus on dietary supplementation for bone health should balance the risks, for example, exposure of vulnerable populations to values close to maximal tolerated doses, against evidence for benefits from randomised clinical trials, such as those for Ca and vitamin D. Feedback from community studies should direct further investigations and help formulate a consensus on dietary supplementation for bone health.

Calcium: Phosphorus: Vitamin D: Vitamin K: Strontium: Magnesium

With the ageing of the population, particularly in Europe, the financial impact on national healthcare systems associated with the management of the diseases of the elderly is expected to increase dramatically in the coming years[1]. Bone diseases will represent a sizable proportion of these costs. The worldwide financial impact of osteoporotic fractures is estimated to double by 2050 to reach 76·7 billion euros[2–4]. In Europe, hospitalisation costs for hip fractures alone currently represent more than 3·5 billion euros each year[3,4].

In 1994, the WHO defined osteoporosis as a disease 'characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to bone fragility and a consequent increase in risk of fracture'[5]. In Europe, it is estimated that 179 000 men and 611 000 women suffer a hip fracture every year[6], and these numbers are expected to double in the next 40 years, as the demographic situation in Europe evolves and the population ages[4]. Although the whole population is at risk, the likelihood of developing osteoporosis increases according to various factors including sex, age, family or fracture history, and Caucasian or Asian ethnic origins[7–9]. Nevertheless, preventive measures are also important since, for example, lack of exercise, smoking, and alcohol consumption have also been identified as risk factors[10,11]. A diet rich in vitamin D and Ca is also recognised as playing a major role in the prevention of osteoporosis[7,12].

Osteomalacia is due to a defect of bone mineralisation resulting in weakened and fragile bones and could also lead to hip fractures[13]. It is the adult equivalent of rickets in

Abbreviations: BMC, bone mineral content; BMD, bone mineral density; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; GluOC, undercarboxylated osteocalcin; 25(OH)D, 25-hydroxyvitamin D; MGP, matrix Gla protein; MK, menaquinone; NHANES III, Third National Health and Nutrition Examination Survey; PTH, parathyroid hormone; RDI, recommended dietary intake; RECORD, Randomised Evaluation of Calcium Or vitamin D; WHI, Women's Health Initiative.

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children and affects approximately 1 in 1000 individuals in Western societies[14]. Like rickets, osteomalacia is mainly caused by insufficient exposure to sunlight uncompensated by the dietary intake of vitamin D[13]. Some defects in vitamin D metabolism or action can also lead to impaired bone matrix mineralisation[13]. Renal diseases and phosphate depletion are also among the major causes of osteomalacia[13]. Maintaining appropriate intakes of vitamin D, if sunlight exposure is insufficient, and Ca constitutes the best prevention strategy.

Nutrition should form part of bone disease prevention strategies[15], especially in the light of the ageing of the population and the effect of diet on bone health. Bone mass peaks at about the age of 18 years and, at skeletal sites such as the proximal femur, it declines thereafter with an acceleration after 50 years of age[7]. The acquisition of bone mineral mass during childhood, adolescence and early adulthood is an important factor determining the risk of osteoporosis in later adult life. Nevertheless, to what extent modifications in environmental factors, such as nutrition and/or physical activity, can influence the risk of osteoporotic fracture in late adhultbood is not known[16].

Because of the influence of cultural environment on nutritional habits, there are very differing opinions on the subject of dietary enhancement and bone health, even regarding the health benefits and safety of the numerous vitamin and mineral supplements available. Although the value of nutrients such as Ca and vitamin D for bone health is well established, a European consensus on maximum levels to allow in food supplementation is still lacking. Moreover, P, vitamin K, Sr and Mg have also been cited as playing a role in bone health. The present paper aims to review the minerals and vitamins that are currently being discussed as part of a supplementation strategy to prevent bone disease. We will therefore discuss Ca, P, vitamin D, vitamin K, Sr and Mg. For each, we examine the bone health benefits, the risks, interactions, and consequences on health, and the normal dietary intake and deficiencies, in order to review the safety and the need for supplementation. As a strategy for finding the most relevant publications we performed a detailed search in the main scientific available databases.

General description of dietary recommendation designations

In the present review, recommended dietary intake (RDI) is used as a generic designation for reference or recommended values for population nutrient intakes. Otherwise, the precise term by the national authority that sets the reference or recommended value is used. In France, RDI for Ca and P, as designated by the term 'apports nutritionnels conseillés', is similar to the former US RDA. In the USA and Canada, the term 'dietary reference intake' was more recently adopted as nutrient reference standards, in replacement of the former Canadian RDI and US RDA. Dietary reference intake includes the 'estimated average requirement', RDA, 'adequate intake' and 'tolerable upper intake level'. As clearly defined by Barr[17], estimated average requirement is the average daily intake level that meets the requirements of 50 % of healthy individuals in a life stage and sex group, whereas the RDA is set at a level that will meet the requirements of almost all (97·5 %) individuals in that life stage and sex group. An adequate intake is a recommended intake level that is thought to meet the needs of almost all healthy individuals, and is set when there are insufficient data to establish an estimated average requirement and therefore an RDA. The tolerable upper intake level represents a threshold above which adverse effects of excessive intake may increase. In the USA, dietary reference intake corresponds to RDA for P and Mg and to adequate intake for Ca, vitamin D and vitamin K. In the UK, 'reference nutrient intake', 'estimated average requirement' and 'lower nutrient reference intake' are used for nutrients with sufficient data to set one recommended level. The term 'safe and adequate intake' is used when too few studies are available to set the other dietary reference values.

Calcium

Bone health and benefits

In the form of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, Ca and P constitute the mineral substance of the skeleton and teeth, which contain 99 % of the adult body's Ca and over 80 % of its P. These two elements therefore play a major role in bone strength and are of prime nutritional importance in osteoporosis[18]. Bone loss can be slowed in the elderly by Ca intake, which reduces the risk of hyperparathyroidism, which in turn promotes bone resorption. The importance of Ca supplementation for bone health is well established and, together with vitamin D, is considered as a key component of any preventive or therapeutic regimen for osteoporosis[18].

The beneficial effect of long-term Ca and vitamin D supplementation on bone and fracture risk in elderly women has also been documented in several clinical trials[18–21]. A recent report from the Women's Health Initiative (WHI) study[22] indicated that prolonged Ca supplementation (plus vitamin D) in healthy postmenopausal women significantly improved bone mineral content (BMD) at the hip. A significant 29 % relative decrease in the risk of hip fracture was recorded, but only among participants who adhered to the Ca + vitamin D supplementation[22]. A large study in the UK, the Randomised Evaluation of Calcium Or vitamin D (RECORD) trial[23] did not find significant anti-fracture efficacy associated with supplementation, but this study had poor compliance and analysed the secondary prevention of fracture. Two recent meta-analyses of randomised controlled trials concluded that additional Ca is needed with vitamin D to reduce the risk of hip fracture and highlighted the importance of compliance with supplementation in reducing fracture risk[24,25].

Nutritional properties. Although Ca has numerous vital functions and is implicated in the incidence of several pathological disorders, the physiological requirements for this element have been principally established on the basis of bone criteria[26–28]. As bone also serves as a hormonally mediated exchangeable reservoir of Ca and P, which is mainly intended to maintain blood Ca at normal levels, it is considered that, by meeting the requirements of bone, the extracellular and intracellular needs of other tissues will also be covered.

Intake from diet. A good proportion of dietary Ca (60–70 %) comes from milk and dairy products. In most European countries[29], cheeses in adults and yoghurts in children and teenagers are particularly important sources of

Ca. With the exceptions of almonds, dried fruit, small bony fish, and a few green vegetables, common foodstuffs are poor in Ca[29,30]. Where flour is fortified with Ca as in the United Kingdom, foods such as bread, biscuits and cakes can make a substantial contribution to total Ca intake. Some mineral waters are rich in calcium bicarbonate, while others are particularly rich in calcium sulfate.

RDI for Ca vary between countries and regions. There is no consensus in Europe, adult RDI varying from 700 mg/d (reference nutrient intake)[27] in the UK to 1000 mg in Austria, Germany, Switzerland and The Netherlands. In France, RDI are 900 mg/d in adults[28] and 1200 mg/d for adolescents, postmenopausal women and the elderly. The American daily adequate intakes are 1000 mg for adults, 1300 mg for adolescents, and 1200 mg for postmenopausal women and the elderly[26].

Necessity to enhance the calcium intake. Recent surveys in France have indicated that mean Ca intake is 1040 mg/d for boys and 820 mg/d for girls between 10 and 18 years of age, 850 mg/d for male adults under 65 years and 790 mg/d over 65 years, 770 mg/d for women under 50 years and 690 mg/d over 50 years, and 500 to 600 mg/d for elderly women in residential care[28]. This suggests that there is a need for enhancing Ca intake, preferably by the consumption of more Ca-rich foods, in teenage girls (200–300 mg/d), postmenopausal women and the elderly (250–300 mg/d), and elderly women in residential care (500–600 mg/d).

These values relate to median intakes in individuals with a diet containing dairy products. However, a diet that does not include dairy products cannot provide more than 400–450 mg Ca/d, unless intake of Ca-rich mineral water is high. In such cases, the need for Ca enhancement is greater and could reach 650 mg/d.

Maximal levels of intake

In France, the upper limit of Ca intake deemed safe when consumed for a prolonged period is 2 g/d, compared with 2·5 g/d in the American recommendations and by the Scientific Committee on Food of the European Commission[31]. This Commission considered that the data were insufficient to derive an upper limit value for children from that of 2·5 g/d set for adults[31]. Hypercalcaemia, alkalosis, and renal and other ectopic calcifications may result if these limits are regularly exceeded over a long period. For example, in the WHI study, in which the average Ca intake was 2·15 g/d (combined with vitamin D) over 7 years in postmenopausal women, there was a 17 % increase in kidney stones[22], though this may have occurred in subjects who were taking additional Ca supplementation, which was allowed in this trial. High doses of Ca can also reduce the absorption of other essential minerals, such as Fe, Zn and Mg[32]. The UK Food Standards Agency did not establish a safe upper limit for total dietary Ca[33], but suggests daily doses of supplementary Ca not higher than 1·5 g/d[34]. A diet rich in dairy products, plus mineral water containing high concentrations of Ca, can provide more than 2 g Ca/d, and a supplement would not then be advisable. In most cases, except with a dairy-free diet, the prescribed daily dose should not exceed 400–500 mg Ca/d for nutritional purposes, which is less than the maximum dose of 800 mg/d adopted in France.

Choice of calcium supplementation

Solubility and bioavailability. The water solubility of the Ca supplement is not a precondition of its biological efficacy, since the intestinal absorption of Ca depends little on solubility in food[35]. The intestinal absorption of Ca from the insoluble calcium carbonate salt is as good as that of Ca from various organic sources and from milk[36–42]. However, solubility should be taken into account for the enrichment of beverages or when supplements are presented as liquids. In the case of powder or tablets, the solubility of the salt is not determinant, except in the extreme cases of highly insoluble salts that are resistant to stomach acids.

Bioavailability in the gut and for bone is the first criterion to take into account when selecting a Ca salt. Good intestinal absorbability is required, but it is only the first step in terms of bioavailability for both bone mineral accretion and bone loss prevention, particularly in the elderly. Some dietary factors influence bone Ca accretion and/or resorption, as well as urinary Ca excretion. Thus, all these factors will determine the overall bioavailability of various Ca salts.

Mineral or organic salts. Various Ca salts are already authorised for the manufacture of food supplements: mineral salts such as carbonate, chloride, oxide, hydroxide and phosphate, and organic salts such as citrate, gluconate, glycerophosphate, pidolate and lactate. Comparative studies have been done on all these salts, and their bioavailability in the intestine is deemed good, at about 25–35 % for moderate intakes, which is of the same order of magnitude as the Ca of milk, which is often taken as a reference[41]. Other Ca salts are the subjects of requests for exemption from Directive 2002/46/EC authorising use in food supplements, inasmuch as they are already marketed for this purpose in the European Community[43].

Several Ca salts or complexes of amino acids, glycinate, L-aspartate, L-lysinate and L-methionate, which are already authorised as sources of amino acids, pose no problem of toxicity and, despite a lack of data in human subjects, the bioavailability of Ca is probably good, but not significantly better than that of mineral salts. The malate (already authorised as a food additive, E 352), pyruvate and succinate salts of Ca are well-known organic acid salts and can be unreservedly authorised. Malic acid is abundant in various fruits and vegetables, and several studies have shown that the bioavailability of calcium citrate-malate is very good, and often greater than that of the Ca of milk[41,44]. The value of a pyruvate or succinate salt is less clear, and no evidence of the safety of a high intake has been published. Other organic sources, such as calcium ethanolamine phosphate and calcium orotate, are probably bioavailable, but nothing guarantees the safety of the anionic moiety, several grams per d of which would be ingested at the doses prescribed for Ca. A Ca chelate of amino acids, principally in the form of bisglycinate, is often presented as a source of Ca with excellent absorbability, notably due to chelation, which protects it until it reaches the site of absorption. Unfortunately, published studies are too scarce to confirm this.

Calcium silicate is authorised as an additive in food technology, but cannot be considered as a source of Ca. It is an inert and insoluble substance whose bioavailability in the gut is certainly very low. Anhydrous or hydrated calcium

sulfate is already authorised in the European Union as a food additive (E 516). The absorbability of the Ca is probably good[45]. However, excess sulfate is likely to increase urinary loss of Ca and therefore decrease the efficacy of its bone retention[46]. In addition, maximum supplementation of 800 mg Ca/d with this salt would provide nearly 2 g sulfate/d, which is close to the laxative dose, particularly as the consumption of sulfate-rich mineral water would raise intake to >3 g/d. Finally, dietary intake of sulfate well below the previous doses appears to be responsible for the production of hydrogen sulfide in the colon by sulfate-reducing bacteria, which can damage the epithelium[47]. The deleterious effects of excess sulfate on urinary loss of Ca and on the epithelial structure of the colon were not taken into account in an approval formulated by the European Food Safety Authority[48]. Before stating that high doses of calcium sulfate are completely safe, it would be advisable to conduct further clinical studies.

Phosphorus

Bone health and benefits

Like Ca, the nutritional requirements for P are determined by bone criteria. The current French RDA are 750 mg P/d in adults and 800 mg P/d in adolescents and the elderly[28]. Similar values are reported in the USA (700 mg/d in all age groups)[26], but lower values in the UK (550 mg/d in adults)[27].

In contrast to Ca, all common foodstuffs (milk, meat, eggs, vegetables and cereals) are naturally rich in P, whose mean intake in Europe ranges from 1300 to 1600 mg/d[28]. P intake may be greater in the case of excessive consumption of foods (for example, ham, processed cheese, surimi) to which phosphate salts, notably polyphosphates, are used as additives. This additional intake of P from processed foods can amount to about 100 mg/d in France, but it can be much higher in the USA[49]. P deficiency is therefore rare in humans and so supplementation would be pointless, or even harmful, except in unusual cases of low-energy diets or the regular use of antacids, which render P insoluble, or in patients with hypophosphataemic osteomalacia. A tricalcium phosphate supplement was shown to be efficacious in the prevention of hip fractures[19], but its main purpose is to supply Ca without decreasing the Ca:P ratio (close to 2 in tricalcium phosphate, compared with just 0·6 in the diet).

Risk, nutritional interactions, and consequences on bone health

Systematic excess of dietary P is inevitable since P content cannot be reduced in common foods[28]. This relative excess of P, compared with Ca, is likely to increase blood phosphate levels, which could lead to Ca metabolism disorders because of a drop in the plasma concentration of ionised Ca, and secondary hyperparathyroidism. This adverse effect of excess P on bone remodelling and on the incidence of osteoporosis has been studied extensively and should not be overlooked[49–52]. Other potential consequences of P excess are ectopic calcifications, particularly in the kidney, diarrhoea, mild gastrointestinal symptoms, and interactions with the intestinal absorption of certain trace elements (Fe, Cu and Zn). P supplementation would then worsen the situation and even sometimes exceed the safety limit of 2·5 g/d adopted in France[28].

Upper tolerable intake levels for P were established in the USA in 1997 at 3 g/d for infants and adolescents and 4 g/d for adults[26]. These levels took into account the observations that P is not toxic, there are few studies showing an effect of a high intake on parathyroid hormone (PTH) secretion associated with bone loss, and PTH can exert an anabolic action on osteoblastic formation. However, these high upper levels have been questioned[28]. Studies indicating the absence of an effect of excess P used only moderate levels (1·6–2·3 g/d) or diets containing sufficient Ca, particularly from milk and cheese[53,54]. Conversely, a high P intake associated with low Ca and/or vitamin D supply gives rise to secondary hyperparathyroidism[49] favouring an increase in bone resorption or an inactivation of the first stages of bone formation[55]. Moreover, excess P decreases renal synthesis of calcitriol[49].

The conclusions of a more recent and extensive European review of the effects of high P intakes[56] did not support the suggestion that induced temporary hyperparathyroidism due to high P intake increases bone resorption, stating that normal healthy individuals can tolerate P intake up to at least 3 g/d without adverse systemic effects. Among the thirteen cited studies concerning the effect of high P intake on serum PTH and bone, only two were less than 11 years old[57,58] and did not report a significant effect. However, the Ca intake in these trials was sufficient for the requirements of young adult men (800 mg/d)[57] or very high (1995 mg/d) for young adult women[58].

According to another expert group of the Food Standards Agency[33,34], using the same published data, changes in PTH levels were found in supplemental studies in postmenopausal women with reduced BMD and a history of fractures, and in healthy men. Changes in plasma Ca and PTH have been associated with intakes ≥ 1500 mg supplemental P/d. The expert group could not establish a safe upper limit for total inorganic P, but estimated that a total intake of 2400 mg/d would not be expected to result in any adverse effect when regularly consumed over a long period. This value is in good agreement with a previous estimation[28] and takes into account all the potential deleterious effects (nephrocalcinosis and gastrointestinal symptoms) as end points to derive an upper level and a no-observed-adverse-effect level of 250 mg supplemental P/d. The maximum daily intake of 450 mg P/d from supplements defined in France corresponds to P combined with a large intake of calcium phosphate with a view to supplying Ca, and could be lowered. Higher levels of P intakes are unnecessary, even for food technology, and should be avoided.

Futhermore, a recent report suggests that higher serum P levels are associated with an increased risk of CVD[59]. This increased risk appears to be unrelated to chronic renal failure or prior CVD. However, it is not established whether this epidemiological association is related to the P intake *per se* or alternatively to an anomaly in the tubular reabsorption of P, the main physiological determinant of the serum P level. Furthermore, since a high serum P concentration leads to lower circulating levels of ionised Ca, which, in turn, will tend to increase PTH secretion, the possible role of

hyperparathyroidism in the incidence of CVD documented in this observational study cannot be ruled out[59].

Vitamin D

Vitamin D has been the focus of much interest and research in the past decade. The roles of vitamin D in Ca absorption and in preventing rickets and osteomalacia in adults are well established. Recent work on methods for vitamin D analysis of foods and serum has enabled more populations to be surveyed for vitamin D status. Surprisingly, vitamin D deficiency and insufficiency are widespread, even at geographical locations near the equator, due to the inability to synthesise adequate vitamin D on exposure to the sun because of dark skin, or habits that limit exposure to UVB radiation. Intervention and dose–response studies have shown health benefits at higher levels than were previously thought to be the upper limit deemed to be safe when exposed for a prolonged period.

Bone health and benefits

Nutritional properties. Vitamin D can come from the diet or from subcutaneous production from 7-dehydrocholecalciferol upon exposure to UVB radiation. There is rapid conversion to cholecalciferol, which is slowly converted to 25-hydroxyvitamin D (25(OH)D) by the liver. Serum levels of this metabolite are considered to be the best indicator of vitamin D status. Renal conversion of small amounts of 25(OH)D to the active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), occurs in response to PTH levels, which increase when serum Ca levels fall or if serum concentrations of inorganic phosphate are low. This form of vitamin D acts on the intestine to enhance active Ca absorption by up-regulating synthesis of Ca transport proteins and on the bone to increase resorption. These actions occur in order to maintain serum Ca levels within the normal range. Without $1,25(OH)_2D$, Ca absorption would occur only by the passive, intercellular route[60].

Although rickets and osteomalacia have long been associated with vitamin D deficiency rather than inadequate production of $1,25(OH)_2D$, there has been a rejuvenated interest in the direct effect of 25(OH)D on bone. Adult serum 25(OH)D levels are more positively associated with Ca absorption efficiency than are serum $1,25(OH)_2D$ levels[61,62], and supplementing with vitamin D or 25(OH)D increases Ca absorption efficiency[63]. In contrast, serum $1,25(OH)_2D$, but not 25(OH)D, predicts Ca absorption in children, as would be expected by the classic model for homeostatic regulation by the vitamin D–PTH axis[64]. Serum 25(OH)D levels have been inversely related to serum PTH levels in both adults[19,65] and children[64,66,67]. In the elderly, increased bone resorption is the main concern with secondary hyperparathyroidism. In children, despite suppression of serum PTH, vitamin D status or supplementation has little impact on bone measures[64,68,69]. One study showed a modest benefit to bone in Lebanese girls given higher levels of vitamin D_3 than in other studies, i.e. 14 000 IU (350 μg)/week, for 1 year[70]. This resulted in significant benefits in gain of total hip bone mineral content (BMC) and area, but not at other sites including femoral neck, trochanter, and total body[70].

Lean mass increased significantly with vitamin D supplementation, which can have an impact on bone through increased muscle mass stress. One study has shown significant advantages of 400 IU (10 μg)/d vitamin D supplementation on bone mineral accretion at the spine and femur in 11-year-old Danish girls[71]. However, another study suggested that larger doses may be necessary to increase BMC in Pakistani immigrant adolescent girls in Denmark[72].

In contrast to children, there is increasing evidence that higher vitamin D status and vitamin D supplementation can positively influence predictors of osteoporosis and fracture in adults. In the Third National Health and Nutrition Examination Survey (NHANES III), serum 25(OH)D was positively associated with hip BMD in 13 432 subjects aged 20 years and older within the reference range of the Diasorin assay for serum 25(OH)D[73]. A meta-analysis of five randomised controlled trials on hip fracture and seven on non-vertebral fracture showed that vitamin D intakes of 700–800 IU (17·5–20 μg)/d reduced the relative risk (RR) of hip fracture by 26 % (RR 0·74; 95 % CI 0·61, 0·88) and non-vertebral fracture by 23 % (RR 0·77; 95 % CI 0·68, 0·87) compared with the control group[74]. Two large trials in the UK, the RECORD trial[23] and one by Porthouse *et al.*[75], and one in the USA, the WHI[22], did not achieve significant anti-fracture efficiency with vitamin D supplementation. This may be due to low vitamin D levels of supplementation in the case of the WHI trial or to poor compliance in the RECORD and WHI trials. The size of the effect with 800 IU (20 μg) vitamin D_1/d in the Porthouse *et al.* trial[75] was similar to that of the meta-analysis[74], but the CI was much wider and included the number 1. The conclusion of two other meta-analyses[24,76] underscored that the beneficial effect on hip fracture prevention required the combination of vitamin D with Ca and appeared to be stronger in frail elderly confined to institutions, as shown in the classical trial by Chapuy *et al.*[19].

Bischoff-Ferrari *et al.*[77] conducted a meta-analysis of randomised controlled trials for the relationship between serum 25(OH)D and the risk of falling. Pooled data from 1237 subjects from five trials showed that vitamin D supplementation reduced the risk of falling by 22 %[77]. Fall reduction may be more pronounced in less active older women, but physical activity and vitamin D may not be related in older men[78]. Assessment of lower-extremity function through functional measures, such as the sit-to-stand test and the 8-foot walk test, have been measured as predictors of fracture risk because muscle function is thought to indicate susceptibility to falls[79,80]. Muscle-specific 1-hydroxylases convert 25(OH)D into $1,25(OH)_2D$ in muscle, and nuclear receptors for $1,25(OH)_2D$ have been identified that lead *de novo* protein synthesis and improved muscle function[74,81,82]. Lower-extremity function increased throughout the reference range of serum 25(OH)D, but especially up to 40 nmol/l, in 4100 ambulatory elderly adults who participated in NHANES III[73]. In Lebanese children, vitamin D supplementation increased accrual of lean mass over 1 year, which explained much of the effect of vitamin D supplementation on total hip BMC and area accrual[70].

Intake from diet. In the absence of adequate exposure to sunlight, the current American dietary reference intake for vitamin D in the form of cholecalciferol is 200 IU (5 μg)/d for adults, rising to 600 IU (15 μg)/d in the elderly

(>70 years)[26]. However, most of the daily input of vitamin D comes from cutaneous synthesis rather than from diet. Moreover, the inadequacy of food composition databases with respect to vitamin D limits an accurate estimate of intake, beyond the usual difficulties individuals experience in accurately remembering and recording their food consumption patterns. Thus, vitamin D intake from diet is not evaluated in most studies.

Natural food sources are mostly fatty fish, fish liver oils, and the fat of fish-eating animals. In countries such as Northern Europe where fatty fish is a major constituent of the diet, intakes of vitamin D have been estimated to be as high as 236 IU (5·9 μg)/d for women and 272 IU (6·8 μg)/d for men[52]. In countries where fortification is prevalent, vitamin D-fortified foods are the major dietary source of vitamin D. Vitamin D status is the highest in countries with mandatory vitamin D fortification, i.e. the USA and Canada for milk and margarine, and lowest in countries with little or no vitamin D fortification (including Europe)[52]. A recent estimate of average vitamin D intakes in the USA from NHANES III was <400 IU (<10 μg)/d[83]. Intakes in UK were estimated as half that in the USA. It could be explained by less use of vitamin D supplements as well as by reduced levels of fortification of dairy products with vitamin D compared with the USA[52]. The levels of fortification in the USA range from 100 IU (2·5 μg) vitamin D per cup (237 ml) of milk or fortified juice to more modest amounts in cereal bars, cheese and other processed foods[84]. Quality control of the levels of vitamin D added to foods has been a problem because of the lack of a quick, reliable test for vitamin D in foods at the site of manufacture and difficulties associated with dispersing and stabilising this fat-soluble vitamin[85].

Necessity to supplement a normal diet. A number of individuals and groups have called for an increase in vitamin D intakes over current recommendations. The criterion for the current requirements and the proposed higher levels of vitamin D intakes are based on adequate serum levels of 25(OH)D. When the current requirements were set, the strategy was to use observed population ranges in serum levels of 25(OH)D. However, serum levels vary with habits that affect sunlight exposure, skin type and colouring, and, to a lesser extent than previously thought, geographical location relative to the equator[86].

More recently, efforts have been focused on optimal serum 25(OH)D levels for various functional outcome measures. For adults, Bischoff-Ferrari *et al.*[78] evaluated the evidence for a threshold serum 25(OH)D level for establishing optimal vitamin D status for multiple bone-related outcomes as outlined above, as well as other health outcomes, and concluded that serum 25(OH)D concentrations should be at least 75 nmol/l, and, better still, between 90 and 100 nmol/l. This level is in line with recommendations of others[87–89], but remains a subject of debate[87]. To achieve this level through diet would require average vitamin D intakes of 1700 IU (42·5 μg)/d[89] and possibly more for dark-skinned individuals[84]. The recent emphasis on dietary sources stems from increasing reports that input from sunlight is becoming inadequate for many populations due to habits that limit sunlight exposure, skin type and pigmentation, and concern about the link between sunlight exposure and melanoma. Clearly, the intakes required to achieve the vitamin D status recommended by some cannot be achieved by diet alone given the current food supply. In fact, such intakes would be difficult to achieve from vitamin D supplements available to date. Only recently have vitamin D supplements been made commercially available that could be used to achieve intakes approaching 2000 IU (50 μg)/d. Calvo *et al.*[52] estimated that dietary supplements in some countries contribute 6 to 47 % of the average vitamin D intakes, and this can be predicted to grow with the increased availability of vitamin D supplements.

Risk, nutritional interactions, and consequences on bone health

Given the low levels of vitamin D in natural foods, dietary vitamin D excesses are rare. Reports of hypervitaminosis D have occurred with accidental overdoses.

The risks of hypervitaminosis D are typically associated with hypercalcaemia arising from a vitamin D-dependent increase in the intestinal absorption of Ca and bone resorption. Adverse effects can include polyuria, pain, conjunctivitis, anorexia, fever, chills, thirst, vomiting and weight loss. Hypervitaminosis D can also lead to calcification of soft tissue[26]. The risk of vitamin D intoxication can be monitored by measuring several biochemical indices to ensure they fall within normal ranges. According to the US Food and Nutrition Board[26], cases of vitamin D intoxication were associated with serum 25(OH)D ranging from 400 to 1250 nmol/l and serum Ca from 2·82 to 4·00 mmol/l, i.e. markedly above the respective normal ranges.

The upper level set by the US Food and Nutrition Board in 1997 was 1000 IU (25 μg)/d for infants and 2000 IU (50 μg)/d for all other ages based on a no-observed-adverse-effect level of 2400 IU (60 μg)/d for hypercalcaemia using evidence in the literature before 1997[26]. The European Commission Scientific Committee on Food also identified an upper level of 2000 IU (50 μg)/d using a similar approach[26]. The UK Expert Group on Vitamins and Minerals set an even lower upper level of 1000 IU (25 μg)/d[33]. Upper levels curtail food fortification, vitamin supplement manufacturers, and policy makers, with legal consequences. Significant additional information has become available since the upper levels were set in 1997 in the USA, notably several dose–response studies. Using the risk assessment methodology for the current upper levels, but with new evidence, Hathcock *et al.*[90] suggested that the upper level for adults could be increased to 10 000 IU (250 μg) vitamin D_3/d. The trials used to propose this level lasted for weeks or months, but not years, and none was conducted in children. Lacking longer-term data and evidence in children creates some uncertainty when considering fortification policies that would affect all individuals.

Vitamin D requirements and safety levels are dependent upon Ca intakes[91]. For bone health, vitamin D input is more important during dietary Ca inadequacy. A recent meta-analysis concluded that vitamin D supplementation required additional Ca to be effective and reduce risk of hip fracture[24]. Risk of hypercalcaemia due to vitamin D excess is greater with high Ca intakes and may lead to increased risk of kidney stones[22]. However, throughout life vitamin D is still essential for bone health even when Ca intake is high.

Maximum levels for supplementation

A requisite for considering increases in levels of fortification of foods or for increases in public policy recommendations for vitamin D intakes is an increase in the upper levels. A vitamin D intake recommendation of 1000 IU (25 μg)/d has been recommended[78]. In the USA, vitamin D fortification is optional in most cases for milk with 100 IU (2·5 μg) per cup (236·5 ml)[92]. The American dietary guidelines suggest consuming three cups of milk daily. Such a diet would provide little more than 300 IU (7·5 μg)/d and the rest would need to come from supplements in the absence of sufficient sun exposure. In this example, that would mean approximately 700 IU (17·5 μg)/d, although some vitamin D would come from other fortified foods. In European countries, supplements would need to provide ≥850 IU (21·3 μg) vitamin D/d. Yet, the benefits of fortification of foods with vitamin D on health outcomes have not been appropriately evaluated in any country. Only small trials in subpopulations have shown the benefit of vitamin D-fortified milk on bone[93,94].

Vitamin K

Rationale for role of vitamin K in bone health

The rationale for a role of vitamin K in bone health originated from the isolation of a vitamin K-dependent protein from bone called osteocalcin (or bone Gla protein) in 1975. This discovery followed the unravelling of the molecular role of vitamin K as an essential cofactor for a post-translational modification, whereby target peptide-bound glutamate (Glu) residues are converted to γ-carboxyglutamate (Gla) residues[95]. Osteocalcin is the most abundant non-collagenous protein of the bone extracellular matrix, and is synthesised by osteoblasts. The central portion of the molecule contains a highly conserved Gla domain that facilitates high-affinity binding to hydroxyapatite. The precise molecular role of osteocalcin is unclear, but it appears to act as a regulator of bone remodelling and mineralisation[96]. Another bone Gla protein, matrix Gla protein (MGP), is a powerful inhibitor of calcification of cartilage. The essential role of MGP in human bone development is demonstrated by the heterogeneous embryopathy, chondrodysplasia punctata, which is characterised by pathological skeletal calcification, including that of the growth plate[97]. The anticalcification activity of MGP depends on the integrity of its five Gla residues, and chondrodysplasia punctata has been shown to be caused by maternal warfarin use or vitamin K deficiency and by congenital MGP mutation[97]. Other Gla proteins present in bone are protein S and Gas6 and the recently discovered periostin[98]. Besides its central role in coagulation, protein S is synthesised by osteoblasts and hereditary protein S deficiency has been associated with osteopenia. Gas6 is known to participate in several cellular processes including bone differentiation and resorption. Periostin is secreted by bone marrow-derived mesenchymal stromal cells and, unusually for a Gla protein, contains four consensus γ-carboxylase recognition sites[98]. *In vitro* studies have shown that carboxylated periostin is localised to bone nodules formed by mesenchymal stromal cells, suggesting a role in extracellular matrix mineralisation[98].

The presence of at least five Gla proteins in bone and cartilage, together with evidence of the essentiality of vitamin K-dependent modification for their function, present a powerful argument for the need for an optimal supply of vitamin K[95,98,99]. We already know that MGP plays an important role in early bone development and that a degree of undercarboxylation of osteocalcin is a common finding in many populations[100], young and old. This suggests that an adequate vitamin K status may be necessary for bone health throughout life. This view is strengthened by recent evidence that periostin, the latest bone-associated Gla protein to be discovered, plays key roles in the processes of bone development and repair[98].

Bone health and benefits

Nutritional properties. Vitamin K is the family name for several biologically active 2-methyl-1,4-naphthoquinones, which possess a variable isoprenoid side chain at the 3 position. Historically, they have been classified as the single plant member, phylloquinone (vitamin K_1) with a phytyl side chain, and a series of bacterial menaquinones (vitamins K_2) with multi-prenyl side chains. The latter are designated menaquinone-n (MK-n) according to the number (n) of prenyl units. The most common bacterial forms have long side chains, typically MK-7 to MK-9. MK-4 is not a common bacterial form, but can be synthesised *in vivo* from dietary phylloquinone. This seems to occur by complete side-chain removal to menadione with subsequent prenylation of this intermediate[101]. Since both phylloquinone and MK-4 are substrates for γ-glutamylcarboxylase, this conversion points to a unique role for MK-4, independent of the recognised coenzyme function of vitamin K.

Different forms of vitamin K have different metabolic properties. For instance, MK-7, a constituent of the ancient Japanese food natto (fermented soyabean), has a longer half-life and greater efficacy for carboxylating osteocalcin than vitamin K_1[102]. More work is needed to determine whether the *in vivo* synthesis of MK-4 means that this isoprenologue has unique actions intrinsic to the geranyl-geranyl side chain.

Intake from diet. The major form of vitamin K in most diets is phylloquinone from plant sources. FFQ-derived estimates of relative intakes of phylloquinone and MK in the Netherlands suggest that about 90 % of total vitamin K intakes are provided by phylloquinone, 7·5 % by MK-5 to MK-10, and 2·5 % by MK-4[103]. In Europe and the USA, at least half of total phylloquinone intakes come from vegetables and vegetable products, of which a quarter to a third comprises green leafy vegetables[104–106]. With the exception of animal livers, the most common dietary source of long-chain MK is fermented foods, such as cheeses and natto.

National surveys reveal considerable variations in phylloquinone intakes within the same population[104–106]. In two UK surveys of older (1994–5)[105] and younger individuals (2000–1)[106] there was an approximate 10-fold difference between the lower 5th and the upper 95th, although the arithmetic means were similar at approximately 80 μg/d. Average intakes in the USA (using similar methodology) are comparable but with a trend to higher intakes in older adults[104]. The UK data also suggest a decline in intakes of phylloquinone in the adult population from 1986 to 2001[106].

Necessity to supplement a normal diet. Current estimates of requirements are based on the coagulation function of

vitamin K rather than on bone health considerations. The current UK 'safe and adequate' intake of 1 μg/kg body weight per d is based on limited dietary restriction studies of the intakes needed to maintain a satisfactory coagulation status and was set in 1991 before any information on dietary intakes was available. It is fortuitous that this adequate intake is virtually identical to average UK intakes[105,106]. The revised adequate intake set by the USA in 2001 on the basis of representative dietary intake data is 120 and 90 μg/d for men and women, respectively[107]. While the US committee noted the interest in new indicators sensitive to vitamin K intake, such as undercarboxylated osteocalcin (GluOC), they did not use them to establish an estimated average requirement because of 'uncertainty surrounding their true physiological significance and the lack of sufficient dose–response data'.

There is a wealth of evidence that a substantial proportion of individuals in the UK and USA fail to meet their national dietary guidelines intakes for vitamin K. In the UK over 50 % of both older[105] and younger adults[106] had intakes below the recommended safe intake and this proportion rises to about 75 % if the benchmark for adequacy is taken as the US adequate intake values.

Population studies in different healthy groups have found that a low dietary consumption of vitamin K is associated with an increased fracture risk[108,109] or a lower bone mass[110], and that an impaired vitamin K status is associated with low bone mass[111] or increased bone turnover[112]. One caveat to these findings is that they are not consistent for the same outcome measures of fractures and BMD, or for age or sex. This may reflect a weak association between vitamin K intakes and bone health, or indicate that certain population groups are more vulnerable to vitamin K deficits than others. Another caveat is that low dietary intakes of phylloquinone could reflect a poor diet in general, and may merely reflect deficiencies of other nutrients important to bone health.

More convincing evidence has come from several epidemiological studies that have found that high circulating GluOC levels constitute an independent risk factor for bone fracture[113–115] and low BMD[116,117]. The strength of these studies is that GluOC is an accepted functional marker of the vitamin K status of the bone matrix and that this association has been found in more than one country with different methodologies for measuring GluOC. This is coupled with the knowledge that increased carboxylation of osteocalcin is readily achievable by dietary supplementation with vitamin K.

Most of the vitamin K intervention studies published to date have been carried out in Japan with high pharmacological doses (generally 45 mg/d) of MK-4 (menatetrenone) as a potential anti-osteoporotic agent. These studies represent a medical intervention rather than nutritional supplementation in patients with pre-existing involutional or secondary osteoporosis or osteopenia. A recent meta-analysis of published, randomised controlled intervention trials with vitamin K suggested a strong association of menatetrenone supplementation with reduced fracture incidence, as well as an effect in reducing bone loss[118]. This meta-analysis also included two positive Dutch trials using phylloquinone at doses of either 10 mg phylloquinone/d in endurance athletes or 1 mg/d in postmenopausal women. The latter 3-year intervention study did not have a vitamin K group alone, but compared a group taking combined vitamins K and D (with Ca and additional minerals) against a group receiving vitamin D with Ca and additional minerals and a group taking a placebo[119]. Only participants taking combined 1 mg phylloquinone, 320 IU (8 μg) vitamin D and minerals showed a slowing of bone loss at the site of the femoral neck, but not at the lumbar spine.

There are two published randomised, controlled supplementation trials in healthy elderly subjects that have employed amounts of vitamin K that are potentially achievable from the diet[120,121]. The first was a 2-year trial carried out in healthy, older Scottish women[120] who were randomised to four groups to receive daily (i) placebo; (ii) 200 μg phylloquinone; (iii) 400 IU (10 μg) vitamin D_3 plus 1 g Ca; or (iv) combined 200 μg phylloquinone and 400 IU (10 μg) vitamin D_3 plus 1 g Ca. This study showed no significant intervention effect on bone loss between groups, but did show a significant increase in BMD and BMC in group (iv) at the site of the ultradistal radius, but not at other sites in the hip or radius[120]. It is of interest that a meta-analysis of studies examining the effects of vitamin K antagonists also showed that the ultradistal site was the bone site that was most responsive to loss of bone after exposure to oral anticoagulants[122]. This effect of vitamin K antagonists seems to mirror the findings of vitamin K supplementation in the Scottish women, albeit in reverse. Overall, the opposing effects of phylloquinone in this Scottish study and the effects of vitamin K antagonists on BMD were both modest. The second study was a 3-year, controlled trial in healthy elderly North American men and women[121] randomised to two groups to receive daily either (i) 500 μg phylloquinone, 400 IU (10 μg) vitamin D_3 plus 600 mg Ca or (ii) 400 (10 μg) vitamin D_3 plus 600 mg Ca. This study showed no effect of the extra 500 μg phylloquinone (taken in addition to recommended amounts of Ca and vitamin D) on bone health as assessed by changes in BMD (femoral neck, spine, or total body) or biochemical markers of bone turnover[121]. There was no difference between the results in men and women.

Risks, nutritional interactions, and consequences on bone health

Concept of differential tissue requirements for vitamin K: mechanistic feasibility considerations. The ability to assess the degree of undercarboxylation of individual Gla proteins in the body has led to the concept of tissue-specific requirements for vitamin K with greater intakes being required to maintain the carboxylation of osteocalcin in bone compared with Gla coagulation proteins in the liver[100]. A key question is to find out whether the epidemiological associations shown for high circulating GluOC and fracture risk can be explained on a mechanistic basis. We are hampered in this regard by a lack of knowledge of the molecular function(s) of osteocalcin. For example, osteocalcin-knockout mice have an increased bone formation, and seemingly stronger bones[123]. However, new evidence suggests that osteocalcin is responsible for subtle changes in the mineralisation process and the maturation of bone mineral crystals that does not show up in most assessments of bone mineralisation[96]. This might explain why some have postulated that vitamin K has a greater effect on bone quality than density, implying that BMD measurements alone may not reflect vitamin K's effects[124].

This view is supported by recent reports that vitamin K improves hip bone geometry and bone strength indices[125,126]. If undercarboxylation of osteocalcin were very harmful, we might expect to see pronounced effects in patients on long-term warfarin therapy. In fact this is not the case, though a meta-analysis suggested an overall detrimental effect of warfarin on BMD[122].

Whether or not GluOC is detrimental to bone health, a substantial and sustained increase in the γ-carboxylation status of osteocalcin is readily achievable by dietary supplementation with nutritionally relevant amounts of phylloquinone. This was clearly seen in the Scottish[120] and American[121] intervention studies in which daily supplementation with 200 and 500 μg phylloquinone respectively resulted in an approximate average 50 % reduction in the fraction of total osteocalcin that was undercarboxylated (%GluOC). The vitamin K_2 member, MK-7, was shown to be more effective than phylloquinone in carboxylating osteocalcin when the same molar dose of 0·22 μmol/d (100 μg phylloquinone; 142 μg MK7) was given to healthy adults for 40 d[102]. Without a mechanistic explanation, however, we do not yet know what degree of osteocalcin carboxylation is optimal, particularly as the use of different methodologies for calculating %GluOC gives very different absolute values.

Nutritional interactions. There is some evidence that vitamin K may act in concert with vitamin D, Ca, and possibly other micronutrients to reduce bone loss. An enhanced effect of combined vitamins K and D was postulated in both the Dutch[119] and Scottish[120] supplementation studies with 1 mg and 200 μg phylloquinone/d, respectively, and has been suggested by the Japanese treatment of osteoporosis with a regimen of MK-4 combined with 1α-hydroxycholecalciferol. It is theoretically feasible that any synergy between vitamins D and K derives from separate effects exerted independently or alternatively from their concerted action on common proteins or pathways; for instance by enhancing the transcription (vitamin D) and post-translational modification (vitamin K) of osteocalcin. Previous epidemiological evidence that vitamin D might directly influence the γ-carboxylation of osteocalcin was not supported by the Scottish study[120].

Maximum levels for supplementation

Although not reflected by current European legislation, vitamin K has a very wide safety margin. This is best illustrated by the widespread use of 45 mg/d doses of MK-4 for the treatment of osteoporosis in Japan. For phylloquinone, regular doses of up to 10 mg/d are used to treat patients with potential malabsorption (for example, cystic fibrosis) or to reverse warfarin over-anticoagulation. In cases of poisoning with superwarfarin rodenticides, injections of 20–50 mg phylloquinone/d may be required over several weeks to stabilise coagulation. In many countries, including the USA, all newborn infants are administered phylloquinone for the prevention of vitamin K-deficiency bleeding.

A potential problem for introducing widespread vitamin K supplementation is the large number of individuals taking oral anticoagulants. The extent of the problem depends on the level of supplementation, but a detailed dose–response study suggested that food supplements providing 100 μg phylloquinone/d do not significantly interfere with oral anticoagulant therapy[127]. A recent paper actually suggests that supplementation with regular daily vitamin K can have beneficial effects on the stability of anticoagulant therapy[128].

Strontium

Bone health and benefits

The possibility of a beneficial effect of stable Sr on bone and in the prevention of osteoporosis was first proposed in 1910 by Lehnerdt[129], who administered strontium phosphate to dogs. However, it was not until the 1950s that human evidence for the benefits of Sr on bone began to appear. First, moderate doses of strontium lactate were shown to improve the deposition of Ca in human bone[130], and later a study in thirty-two patients receiving an oral dose of 1·7 g strontium lactate/d reported increased bone mass on X-ray in 78 % of cases[131]. Several animal and cell-culture studies documented that Sr, particular strontium ranelate, can exert a positive effect on bone formation, while inhibiting bone resorption[132–136]. Strontium ranelate was demonstrated to have both anti-resorptive and bone-forming activity, and was predicted to be of benefit in the treatment of bone diseases[137]. The therapeutic benefits of oral administration of 2 g strontium ranelate/d in postmenopausal osteoporosis were confirmed in 2004 when a large placebo-controlled clinical trial in 1649 women reported early (after 1 year) and sustained (over 3 years) significant reduction in the risk of vertebral fracture[138]. Strontium ranelate's anti-fracture efficacy was shown to extend to non-vertebral fracture in 2005, following publication of the results of a placebo-controlled study including 5091 women with postmenopausal osteoporosis[139]. Treatment with 2 g strontium ranelate/d significantly reduced the risk of non-vertebral and hip fracture over 3 years[139]. The vertebral, non-vertebral, and hip anti-fracture efficacy has recently been confirmed over 5 years[140]. The efficacy of Sr in the primary and secondary prevention of osteoporosis fracture was obtained with quantities of up to 680 mg/d corresponding to 2 g strontium ranelate/d, and a daily intake of 0·13 mmol Sr/kg per d. All the reliable human data on Sr were conducted with strontium ranelate under medical supervision in a specific population with postmenopausal osteoporosis[138–141]. This agent is now recognised as a drug treatment for osteoporosis in Europe.

The question of bioequivalence of the various Sr salts remains unclear. Bioavailability in animal experiments appears to vary from 5 to 25 % of the ingested dose under normal physiological conditions[142], but comparative human absorption data are incomplete and the relative beneficial effects on bone unknown. The potential bone benefits for the general population of oral supplementation with Sr have not been assessed. It is unclear whether the benefits of strontium ranelate extend to other salts. Further evaluations including broader populations and other salts of Sr are necessary.

Intake from diet. The average intake of Sr in a normal diet is generally accepted to be between 1 and 2 mg/d in Western populations[143]. Consumption of vegetables grown in Sr-rich soils may increase intake[144]. Sr concentrations in drinking water may vary from one geographic region to another, but are generally agreed to lead to an intake between 2

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and 4 mg/d, considering that humans should drink about 2 litres/d[145]. Although Sr is found in practically all foods, the concentrations remain very low. The highest concentrations are found in fish, seafood, nuts and leafy vegetables[143,146]. Other vegetables, cereals and fruit also contain significant amounts of Sr, while meat and eggs have very low concentrations[147].

Necessity to supplement a normal diet. Sr does not have any recognised biological role essential to health[148]. No requirement for dietary supplementation with Sr has been established to date. None of the national and international organisations mentions Sr as an essential nutrient or as part of a nutritional strategy to improve bone health[2]. No RDI has been established because the decision to supplement a normal diet must be made on the basis of observed deficiencies in humans and no such cases have been identified for Sr. The logical conclusion is that there is no need for supplementation in the general population, even for prevention of fracture in postmenopausal osteoporotic women for whom the strontium ranelate salt is currently used as a treatment.

Risks, nutritional interactions, and consequences on bone health

Although Sr is not toxic to humans, a number of animal studies have demonstrated the detrimental consequences of high doses of Sr (>4 mmol Sr/kg per d)[149]. Such doses of Sr were shown to induce skeletal abnormalities[129,150]. Most of these interactions concern Ca competition at the bone[151] or intestinal level[152]; these interactions could induce hypocalcaemia[152]. At elevated levels, Sr replaces Ca in bone and can lead to the inhibition of calcification of epiphyseal cartilage and long-bone deformities in growing rats[151,153]. Oral administration of excess Sr in rats inhibited the increase in Ca intestinal absorption normally stimulated by vitamin D[154]. The carbonate, gluconate and chloride salts of Sr have also been reported to inhibit vitamin D activity in rats[136], leading to bone malformation, while Sr ions have been reported to produce rickets in rats by inhibiting the renal production of vitamin D[155].

In humans, the induction of 'Sr rickets' has been observed in children ingesting a low-vitamin D diet for 11 months living in two different regions of Turkey with different soil concentrations of Sr[144]. The proportion of children with rickets was significantly higher in the region with Sr-rich soil than in the region with Sr-poor soil[144]. This indicates the possible importance of the effect of environmental Sr on bone health, and illustrates how some populations could be at high risk if Sr supplementation were implemented.

Interactions of Sr ions with lactose may increase the absorption of Sr[156]. It has been suggested that this may present a risk in overdose[150] since lactose decreases the discrimination of Sr and Ca during intestinal absorption, particularly in children[150,153,157]. The vulnerability of children is reinforced by the fact that the body burden of young children is greater than in adults and so the bone consequences of Sr overdose would be more severe[148]. Raised levels of Sr have been shown to have adverse effects in the bone of offspring of pregnant or lactating animals[158,159], suggesting that Sr should not be supplemented in pregnant or lactating women. In the presence of impaired kidney function Sr supplementation should also be avoided, since it could lead to osteomalacia as documented in a rat model of chronic renal failure[160].

Although the detrimental effects of Sr only appear at elevated levels, some populations may be at risk if they ingested supplementary Sr without proper medical supervision. Nevertheless, perusal of the literature reveals how little is really known about Sr and its toxicity, and the studies on the element are not always reliable[148]. Apart from the studies into the therapeutic applications of strontium ranelate[138,139], there are no studies investigating the safety of long-term oral exposure to other stable Sr salts in humans.

Magnesium

Mg is a nutritional bone component that has been poorly studied for its role in bone health. Because bone undergoes continuous remodelling, an adequate supply of Mg, as well as other nutrients important to bone, is needed to support bone formation. As with Ca, there is a potential risk of dietary deficiency for Mg. Studies have shown that Mg deficiency could affect bone growth and osteoblastic and osteoclastic activity, induce osteopenia and bone fragility, and alter Ca metabolism by affecting calciotropic hormones[161]. Note that there are no reliable biomarkers of Mg status or function. RDI have been set on average intakes or balance estimates that may not, therefore, reflect biological requirements.

Bone health and benefits

Nutritional properties. Approximately 60 % of the Mg in the body is in bone. Mg influences mineral metabolism indirectly through its role in ATP metabolism and as a cofactor for over 300 proteins, the calciotropic hormones and $1,25(OH)_2D$. Mg also influences bone health by direct effects on bone quality, decreasing hydroxyapatite crystal size, thereby preventing the larger, more perfect mineral crystals that could lead to brittle bone. Further, Mg also can indirectly influence bone by acting as a buffer for the acid produced by the typical Western diet, since Mg is found in green, leafy vegetables, legumes and whole-grain products, nuts, seeds, fish and hard water. Recent studies have found a positive association between fruit and vegetable consumption and bone health[162–165].

The scant clinical data available would support a bone health benefit with dietary Mg. Small epidemiological studies have found positive associations between Mg intake and BMD. Preadolescent Mg intake is positively related with bone mass in young women[166]. High Mg intakes have been associated with higher BMC in Australian women[167], higher BMD at different sites in Scottish menopausal women[163,168], Italian and American white postmenopausal women[169,170], and elderly American white men and women[162,171]. In addition, higher Mg intakes have been found to be linked with lower bone resorption markers in Scottish women[163,168].

Clinical studies with Mg supplementation have also found a positive impact on bone. Supplementation with 300 mg Mg/d for 1 year (total Mg intake including diet, 484 mg/d) significantly increased hip BMC in white adolescent girls[172]. Supplementation with 600 mg Mg/d (plus 500 mg Ca/d) for 0·5 to 1 year resulted in mean increases of 11 % in

BMD in postmenopausal women, which persisted at 2 years' follow-up[173]. Mg supplementation with 250 to 750 mg/d (total Mg intake, 450–1050 mg/d) for 1·5 years resulted in gains in BMD in most postmenopausal women, with no reported side effects[174]. In addition, Mg supplementation of 365 mg/d for 30 d (total Mg intake, 665 mg/d) reduced bone turnover in young men[175].

Intake from diet. American RDI for Mg were increased in 1997 to 310 and 400 mg/d in younger (aged 19–30 years) female and male adults, respectively, and to 320 and 420 mg/d in female and male adults aged over 30 years[26]. The European Union population reference intake for adults is 150 to 500 mg Mg/d, and the European Commission RDI is 300 mg/d[176].

The primary source of Mg is food, but consumption from supplements is increasing due to the addition of Mg to many commercially available products. Population studies in the USA report low Mg intakes of 326 and 237 mg/d in white men and women, respectively[177]. In Europe, most countries report an intake >300 mg/d in men, except in Italy, which is below this level[178]. In women, Mg intake is about 300 mg/d, except in Italy, France and Spain; in the elderly, intake is below this level in most countries. In contrast, Mg intake is high in Germany, Denmark and Hungary (about 416 mg/d).

Uncertainty regarding the necessity to supplement a normal diet for bone health. According to the US RDI, Mg intake would be suboptimal in most individuals, specifically women from the southern European countries and the elderly. This is due to an increased consumption of overly refined and/or processed cereal-crop and carbohydrate food staples, which removes most of the Mg.

On the basis of these observations, one may infer that diets should be supplemented with Mg. However, a recent French study reported that recommending Mg supplements would only increase Mg intake by 10 %[179]. Therefore, supplementing food with Mg would appear to be a better strategy to increase Mg intake to RDI levels in countries with relatively low spontaneous intake. Whether such a food fortification with Mg would improve bone health is not established.

Risk, nutritional interactions, and consequences on bone health

Mg excess from a normal diet is rare. Studies of vegetarians in Sweden and the USA report high Mg intake (440–615 mg/d), with no associated health risks[180,181]. Mg in excess or deficit in rats (levels much below or above 0·25 % dietary Mg) resulted in bone of abnormal appearance and chemical composition[182]. Mild diarrhoea is the most undesirable effect of orally administered easily dissociable Mg salts. No laxative effects have been observed for Mg in adult men and women, even during pregnancy and lactation, or in children aged over 4 years, at doses up to 250 mg/d, which is considered as the no-observed-adverse-effect level. No other adverse health effects have been reported with Mg supplementation, even at levels as high as 1000 mg/d.

As shown in human studies, there are significant interactions between Mg and the intestinal absorption of Fe and Zn, probably owing to the inhibition of gastric acid by Mg[183–185]. Interactions with drugs such as tetracyclin, penicillin and digoxin have also been reported[186]. It has been suggested that high-Ca diets could intensify Mg deficiency; but short- and long-term studies of Mg balance do not support this notion[187–189].

Maximum levels for supplementation

Assuming that future research will firmly establish the need for dietary Mg enhancement for bone health, various amounts have already been proposed. Former reports recommended Mg supplementation as high as 600 mg/d for the elderly, regardless of Mg status[173,174]. A safe model for Mg addition to foods has been developed in Europe, which proposes a maximum level of 240 mg/d to be added to foods[176]. If 10 % of all fortifiable foods were fortified with Mg, 10 mg added to 100 kcal (418·4 kJ) of food portion will provide 200 mg Mg/d in a 2000 kcal (8368 kJ) diet. This would increase usual dietary Mg intake from 200–300 mg/d to 400–500 mg/d, in women and in the elderly, and to >500 mg/d in men.

Conclusion

Although the value of supplementary Ca and vitamin D in bone health is well established, there is a distinct lack of consensus regarding the other vitamins and minerals implicated in bone health. Ca and vitamin D supplementation is recommended in postmenopausal women and the elderly, and in cases of osteoporosis. However, Ca supplementation is recommended to the extent that the diet is poor in milk and dairy products. The benefits of such a programme of supplementation is supported by the results of randomised controlled trials. Vitamin D is available in the diet from some foods, but most comes from cutaneous synthesis and serum levels may vary according to individual exposure to sunlight. Current guidelines on vitamin D supplementation may not have taken into account recent evidence, and underestimate desirable intakes and maximal levels. Although P is essential to bone health, supplementation is not recommended since Western diets are almost systematically overgenerous in terms of P content due to the high natural content of most common foodstuffs and its widespread use as an additive in food technology. Vitamin K appears to enhance the actions of Ca and vitamin D, and may be important to bone health. However, there is no agreement on maximal tolerated levels, especially in individuals taking oral anticoagulants; whether the general population would benefit from vitamin K supplementation for bone health remains unclear. As regards Sr, in contrast to the other minerals and vitamins, there is no published nutritional requirement, no upper level has been defined, and there is no established lower serum concentration at which pathological symptoms appear. One Sr salt, strontium ranelate, is currently authorised for the treatment for postmenopausal osteoporosis, but there is no evidence that Sr supplementation would be beneficial to bone health in the general population. Mg is essential for skeletal health. Nevertheless, it is not firmly established that there would be a risk in the general population of dietary Mg deficiency leading to increased bone fragility. Therefore, despite the fact that in the Western diet Mg is generally lower than recommended intakes, current evidence is not strong enough to promote enhancement of this element via fortification of foodstuffs.

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