

## The roles of vitamins D and K in bone health and osteoporosis prevention

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Vitamins D and K are two fat-soluble vitamins that play different but essential roles in bone metabolism. Vitamin D, after its conversion to a dihydroxylated metabolite, acts as a classical steroid hormone that regulates the transcription of manifold target genes involved with Ca homeostasis in general and bone mineralization in particular. The role of vitamin D in promoting mineralization of bone has of course been appreciated for many years, together with the deficiency syndromes of rickets and osteomalacia that result from prolonged deficiency states in childhood and adulthood respectively. Vitamin K, on the other hand, is a cofactor for a carboxylase enzyme that resides in several tissues and promotes the post-translational carboxylation of a number of specialized proteins with Ca-binding properties. They include at least three osteoblastic bone proteins whose functions remain unclear. The synthesis of the best studied vitamin K-dependent bone protein osteocalcin is also regulated by vitamin D. This protein is also one of the ten most abundant vertebrate proteins and represents an interesting point of convergence of the different actions of vitamins D and K.

Osteoporosis is a condition characterized by a low bone mass and microarchitectural deterioration of bone tissue that results in an increased bone fragility and increased fracture susceptibility. Neither the nature of osteoporosis (Marcus, 1996) nor its relationship to nutrition in the broad sense (Heaney, 1996; Prentice, 1997) will be considered here. Instead the nutrition and biochemistry of vitamins D and K will be outlined, followed by a consideration of the possible roles of each micronutrient in the pathogenesis and prevention of osteoporosis. If osteoporosis is viewed as being inseparably bound to the normal ageing process, then in the context of the present article it is obviously important to consider the consequences of any ageing process to the nutrition and metabolism of vitamins D and K. There is evidence of age-related changes for both vitamins that may be relevant to osteoporosis. Finally, an important question to consider is whether nutritional or pharmacological supplementation with vitamins D and K may prove to be of benefit to the treatment and prophylaxis of osteoporosis.

One potentially exciting area will be omitted and this is the question of gene–nutrient interactions and particularly how polymorphisms of the vitamin D-receptor (VDR) gene might affect bone mineral density (BMD). The main reason for this omission is that the results in this rapidly-moving field are presently in conflict (Houston *et al.* 1996). Another intriguing hypothesis linking lipoprotein-mediated vitamin K transport and polymorphisms of apolipoprotein E to bone integrity also needs further work (Kohlmeier *et al.* 1996).

### VITAMIN D: SOURCES OF VITAMIN D

#### *Photosynthesis of vitamin D in human skin*

The importance of sunshine exposure for vitamin D synthesis by the skin is well known. Less well known, until the recent work of Holick's group in Boston, have been the quantitative aspects of the skin photochemistry and the associated environmental factors that together have a practical impact on vitamin D status (for review, see Holick, 1995). As

much as 80–90% of the body's stores of vitamin D may come from casual exposure to sunlight. However, in northern climes, photoproduction in the skin is a very seasonal phenomenon limited to the summer months. Thus, only u.v. B photons with energies between 290 and 315 nm are able to penetrate the skin and reach the epidermal and dermal stores of 7-dehydrocholesterol (procholecalciferol). 7-Dehydrocholesterol is both the immediate precursor for cholesterol (so the skin stores are plentiful) and is photolabile. It is the latter property that is harnessed by the skin and results in the photolytic bond splitting of 7-dehydrocholesterol to precholecalciferol. Precholecalciferol is unstable and undergoes a membrane-enhanced isomerization to cholecalciferol that binds to the vitamin D-binding protein and enters the circulation. In northerly latitudes the quantitative conversion of 7-dehydrocholesterol to precholecalciferol has been shown to decline markedly in winter such that in Boston, USA (42°N) there is no detectable production of precholecalciferol between November and February (Holick, 1995). As discussed later, the seasonality of vitamin D status in places well away from the equator may have important consequences to bone health.

#### *Dietary sources*

The only rich dietary sources of vitamin D as cholecalciferol are fatty fish such as herrings (*Clupea harengus*), mackerel (*Scomber scombrus*), pilchards (*Sardina pilchardus*), sardines and tuna (*Thunnus albacares*), but these are rarely major contributors to the diet. Other useful sources are eggs and, depending on the country, fortified foods such as milk, yoghurts, margarines and breakfast cereals. The other naturally-occurring form of vitamin D, ergocalciferol, occurs in plants and fungi (produced by u.v. irradiation of ergosterol) but makes little impact as a dietary source except where this compound is used instead of cholecalciferol to fortify foods. The average daily dietary consumption of vitamin D in the UK is about 3 µg (Ministry of Agriculture Fisheries and Food, 1994) and similar intakes pertain to the USA (Dawson-Hughes *et al.* 1995). In the UK, current recommendations (Department of Health, 1991) are that only people aged over 65 years need an extra dietary source (10 µg daily) but this is under review.

### METABOLISM AND ACTIONS OF VITAMIN D

#### *Metabolic events leading to the biologically-active form of vitamin D*

Whether derived from skin synthesis or the diet, vitamin D only becomes biologically active after two sequential tissue hydroxylation reactions. The first hydroxylation reaction is carried out by the liver and results in the production of the major transport form 25-hydroxyvitamin D (25 (OH)D) while the second is carried out by the kidney and results in the active hormonal form 1,25-dihydroxyvitamin D (1,25 (OH)<sub>2</sub>D). The same metabolic hydroxylations apply to both cholecalciferol and to ergocalciferol, but normally the major hydroxylated metabolites in the body are 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol, reflecting the predominance of skin-produced cholecalciferol as the original source. Once formed in the kidney, 1,25(OH)<sub>2</sub>D is re-secreted into the circulation and is delivered to those target tissues with cells that possess a specific nuclear VDR. In each target cell, the 1,25(OH)<sub>2</sub>D binds to the VDR to form a complex that binds to regulatory sequences in the upstream region of genes that are regulated by 1,25(OH)<sub>2</sub>D (Hannah & Norman, 1994). The major target tissues are the intestine and bone where the biological responses to 1,25(OH)<sub>2</sub>D are an increase in Ca absorption and Ca mobilization respectively.

*Biological actions of 1,25-dihydroxyvitamin D on bone mineralization and remodelling*

The best known and perhaps most important physiological means by which vitamin D affects and maintains bone mineralization is to ensure adequate concentrations of Ca and phosphate in the circulation and extracellular fluid space. This is demonstrated by the correction of the mineralization defect in a child with hereditary resistance to  $1,25(\text{OH})_2\text{D}$  by long-term, nocturnal infusions with Ca and phosphate (Balsan *et al.* 1986) and by several similar studies in vitamin D-deficient rats (see Bikle, 1994; Holick, 1996).

$1,25(\text{OH})_2\text{D}$  also has direct effects on bone *in vitro* that may be of physiological consequence to bone mineralization. Unravelling the direct effects of  $1,25(\text{OH})_2\text{D}$  on bone remodelling is complicated by its apparent action on several cell types and the fact that the response is dependent on the stage of differentiation of the bone cell (Feldman *et al.* 1996).

The bone-forming osteoblasts express the VDR and  $1,25(\text{OH})_2\text{D}$  regulates the synthesis of several osteoblast gene products that play a role in bone matrix formation and mineralization. These include collagen, alkaline phosphatase (EC 3.1.3.1), osteopontin and the two vitamin K-dependent proteins osteocalcin and matrix Gla protein (MGP). The level of expression of gene products such as osteocalcin that are regulated by  $1,25(\text{OH})_2\text{D}$  can range from down-regulation in proliferating immature osteoblasts to up-regulation in mature osteoblasts (Lian & Stein, 1996). The wide-ranging capacity of  $1,25(\text{OH})_2\text{D}$  to induce differentiation is seen by its ability to stimulate the differentiation of osteoblasts from immature, proliferating cells to fully-differentiated, non-dividing cells that possess the capacity to synthesize matrix proteins and to mineralize bone.

A well-established humoral effect of  $1,25(\text{OH})_2\text{D}$  on bone is the stimulation of osteoclast-mediated bone resorption both *in vivo* and *in vitro* (Teitelbaum *et al.* 1996). When  $1,25(\text{OH})_2\text{D}$  is added to organ cultures there is an increase in bone resorption accompanied, amongst other effects, by an increase in osteoclast number and activity. Apparent paradoxes of this effect are that isolated mature osteoclasts fail to respond to  $1,25(\text{OH})_2\text{D}$  unless co-cultured with osteoblasts and that fully-differentiated osteoclasts do not possess a VDR. The increase in resorptive activity of mature osteoclasts associated with  $1,25(\text{OH})_2\text{D}$  is thought, therefore, to be indirect and mediated via the osteoblast.

Besides promoting the activation of osteoclasts,  $1,25(\text{OH})_2\text{D}$  also promotes their differentiation from mononucleated precursors. This action of  $1,25(\text{OH})_2\text{D}$  on osteoclastogenesis is complex and the steroid hormone regulates both osteoclast precursors and modulatory bone-marrow stromal cells (Suda *et al.* 1992; Teitelbaum *et al.* 1996).

## ASSESSMENT OF VITAMIN D STATUS

The simplest and most widely used biochemical marker of vitamin D status is the level of circulating  $25(\text{OH})\text{D}$ . Major advantages are that  $25(\text{OH})\text{D}$  is mainly confined to the extracellular compartment, circulates tightly bound to the vitamin D-binding protein and has a very long half-life. As the first metabolite formed in the liver from vitamin D, the plasma pool of  $25(\text{OH})\text{D}$  represents a stable reservoir of a metabolite that lies between the synthesis or intake of vitamin D and its utilization as a hormone. Although responsive to the balance between synthesis or intake and utilization, like other vitamin assays it is difficult to define precise cut-off points to differentiate between a normal and abnormal vitamin D status (Bouillon, 1990; Lips, 1994). A major complication in defining population reference values is the dependence of serum  $25(\text{OH})\text{D}$  on sunshine exposure resulting in geographical and seasonal variability. For individuals it is obviously more straightforward in the clinical arena to define an overt deficiency that will lead to rickets or osteomalacia

than to define or detect a sub-clinical vitamin D-deficient state that may over a prolonged period of time exert more subtle adverse effects on the skeleton.

#### VITAMIN D AND OSTEOPOROSIS

In a review of vitamin D in bone health in the elderly, Parfitt *et al.* (1982) wrote 'Potentially much more important from the standpoint of public health (i.e. than osteomalacia) is the question of whether sub-clinical vitamin D deficiency can play a role in the pathogenesis of osteoporosis in the elderly and so contribute to the increasing incidence of hip fractures'. They went on to state 'It is surprising how often this possibility is ignored'. In discussing the mechanisms of how this might occur, Parfitt *et al.* (1982) highlighted the relationship between vitamin D deficiency and secondary hyperparathyroidism in the elderly that they argued could cause accelerated loss of bone by increasing bone turnover. Fifteen years later on, with our correspondingly greater understanding of the interplay between nutritional, metabolic, and genetic factors that together govern the supply and effectiveness of vitamin D in exerting its biological actions on target tissues, there is a much greater appreciation of the pathogenic role of vitamin D deficiency in osteoporosis and how this may indeed be mediated through parathyroid function (see Table 1).

Vitamin D plays a central and lifelong role in bone remodelling and there is now good evidence that age-associated changes in several aspects of its synthesis, absorption, and metabolism may be deleterious to bone health. Individually such changes may be considered relatively minor and often may be a normal consequence of ageing. However, when working in concert over many years, they may lead to significant bone losses, either directly or indirectly by causing alterations in an interacting system one or more steps removed. These changes and their consequences are now discussed in detail.

#### *Effect of ageing on vitamin D status*

Measurements of 25(OH)D in population studies have been useful in studying the determinants of vitamin D status and how this may change with ageing. Lund & Sørensen (1979) found that 25(OH)D levels were significantly higher in younger (18–60 years)

Table 1. *Possible sequence of events from suboptimal vitamin D status to osteoporosis and exacerbating factors in elderly subjects*

Event	Leads to	Consequence
Sunshine exposure or dietary vitamin D intake↓	Serum 25(OH)D↓	Impaired vitamin D status
Serum 25(OH)D↓	Serum 1,25(OH) <sub>2</sub> D↓	Deficiency of active hormone
Deficiency of active hormone	Ca absorption↓ (Serum Ca↓ serum P↑)	Negative Ca balance
Negative Ca balance	Parathyroid hormone↑	Secondary hyperparathyroidism
Secondary hyperparathyroidism	Bone turnover↑	Bone loss↑ and osteoporosis
Exacerbating factors in elderly		
Impaired skin synthesis of vitamin D		
Impaired renal function and/or specific renal synthesis of 1,25(OH) <sub>2</sub> D		
Decreased number of vitamin D receptors and/or organ resistance		

25(OH)D, 25-hydroxyvitamin D; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; ↑, increase; ↓, decrease.

compared with older (61–93 years) subjects but the correlation between 25(OH)D and age was only seen in the summer months.

There are several reasons why the elderly may have reduced circulating levels of 25(OH)D that may be indicative of a suboptimal vitamin D status. One reason is that the elderly, voluntarily or involuntarily, generally have less exposure to sunlight. Another reason is that there is a marked age-dependent decrease in the epidermal concentrations of the vitamin D precursor, 7-dehydrocholesterol (MacLaughlin & Holick, 1985). The ensuing decreased synthesis of vitamin D has been shown experimentally by comparing plasma vitamin D levels in young (22–30 years) and elderly (62–80 years) subjects in response to the same dose of skin irradiation (Holick *et al.* 1989). Maximal concentrations of vitamin D occurred after about 24 h and were threefold lower in the older group than the younger group (22 v. 78 nmol/l). Although a reduction in skin synthesis is probably the most important reason for the lowered vitamin D status of the elderly (Compston, 1995), this may be exacerbated by a less-efficient intestinal absorption of vitamin D (Barragry *et al.* 1978) and by lower dietary intakes.

Even though the elderly may have a reduced capacity for vitamin D synthesis, they still are able to increase their tissue stores and, hence, benefit from regular sunlight exposure. In a study from Auckland, New Zealand, elderly residents of a rest-home received 15 or 30 min of spring sunlight exposure to the head, neck, forearms and lower legs daily (Reid *et al.* 1986). Circulating levels of 25(OH)D rose progressively in both groups and at the end of the 4-week period of study the mean level in the 30 min/d group had risen by about 30 %.

#### *Effect of ageing on vitamin D metabolism and action*

Of the two sequential hydroxylation reactions of vitamin D to 25(OH)D and 1,25(OH)<sub>2</sub>D, only the latter is influenced by age. The possibility of an age-related decline in renal production of 1,25(OH)<sub>2</sub>D was first indicated by the study of Slovik *et al.* (1981) who showed that the ability of elderly osteoporotic patients to produce 1,25(OH)<sub>2</sub>D in response to parathyroid hormone (PTH) was markedly impaired. In a later PTH response study, specifically designed to test age effects, Tsai *et al.* (1984) produced further evidence that the ability of the kidney to synthesize 1,25(OH)<sub>2</sub>D declines with age. This decline in renal hydroxylating capacity is entirely compatible with an age-related decline in renal function rather than a specific defect in the enzymic activity of the 25(OH)D 1 $\alpha$ -hydroxylase (EC 1.14.13.13) *per se* (Silverberg *et al.* 1996).

In addition to the decreasing ability with age to synthesize 1,25(OH)<sub>2</sub>D, there is evidence for an intestinal resistance to the action of this hormone (Eastell *et al.* 1991). The mechanism of this intestinal resistance is unclear but could be due to an age-related decrease in the concentration of intestinal VDR as supported by measurements in intestinal biopsies from women (Ebeling *et al.* 1992).

#### *Hypotheses relating vitamin D deficiency or malfunction to osteoporosis*

Several lines of evidence suggest a role for suboptimal vitamin D status as a possible contributory cause of bone loss in the elderly. Simple measurements of serum levels of 25(OH)D or 1,25(OH)<sub>2</sub>D are of limited value in defining vitamin D-deficient states because of the complexity of their interpretation. Measurements of 25(OH)D require carefully-controlled assays and reference values that are appropriate for the season and population. When such criteria are met, Lips (1994) noted a progressive downward trend in 25(OH)D



levels in four groups from healthy young adults, to elderly people living independently, to institutionalized elderly, and elderly patients with hip fractures. A similar general trend to low levels of 25(OH)D in the elderly and osteoporotics have been found by others. The interpretation of 1,25(OH)<sub>2</sub>D levels is more complex, being normally under tighter metabolic control and reflecting several influences such as vitamin D status, renal 25(OH)D 1 $\alpha$ -hydroxylase activity, PTH activity and physical mobility. This complexity of influence may explain the lack of consensus with respect to circulating 1,25(OH)<sub>2</sub>D in fracture patients, with some studies reporting low levels and others finding normal levels (Reid, 1996). This is further illustrated by vitamin D supplementation studies in the elderly that always result in large increases in circulating 25(OH)D but may elicit no increase (Himmelstein *et al.* 1990; Chapuy *et al.* 1992; Heikinheimo *et al.* 1992), or only a slight increase (Lips *et al.* 1988), in circulating 1,25(OH)<sub>2</sub>D. Further analysis of this response showed that the increase in 1,25(OH)<sub>2</sub>D was inversely related to the initial 25(OH)D level, presumably because only in vitamin D deficiency is the synthesis of 1,25(OH)<sub>2</sub>D substrate-dependent (Lips *et al.* 1988).

More convincing evidence for the role of sub-clinical vitamin D deficiency in osteoporotic bone loss comes from studies linking vitamin D status with parathyroid function and bone density. Seasonal variations and inverse correlations between serum PTH (highest in winter) and serum 25(OH)D (highest in summer) are known to occur in healthy post-menopausal women (Krall *et al.* 1989) and in healthy elderly people (Hegarty *et al.* 1994). Krall *et al.* (1989) showed that this inverse relationship between PTH and 25(OH)D was dependent on dietary vitamin D intake and that daily intakes of at least 5  $\mu$ g were needed to offset the wintertime increase in PTH. Intervention studies with higher daily intakes of vitamin D have also shown that parathyroid function may be suppressed in elderly subjects by improving their vitamin D status (Chapuy *et al.* 1987; Lips *et al.* 1988). The hypothesis that these changes are significant to bone is strengthened by studies showing that bone density is positively related to serum 25(OH)D and inversely related to serum PTH (Khaw *et al.* 1992; Rosen *et al.* 1994). As shown by Rosen *et al.* (1994) there is a strong seasonal component to these variables with an overall decline in BMD in winter (with lowest serum 25(OH)D and highest PTH) and a modest increase in summer (with highest serum 25(OH)D and lowest PTH). A negative relationship between PTH and BMD has also been found in healthy elderly men (Murphy *et al.* 1993).

Although the evidence outlined previously points to a role for vitamin D deficiency or altered metabolism in the pathogenesis of osteoporosis, the relative importance of the various factors shown in Table 1 is as yet unclear. The beneficial effect of vitamin D in osteoporosis has usually been thought to be mediated through improved Ca absorption. However, it is at least feasible that an impairment of other actions of vitamin D on bone metabolism may be important. An alternative hypothesis is that the alterations in the vitamin D system are not in themselves causative of bone loss but induce alterations in another system such as parathyroid function (Silverberg *et al.* 1996). This view, whereby PTH is seen as a culprit that induces bone loss in the vitamin D-deficient elderly by increasing bone turnover, has gained ground in recent years (Lips, 1994; Compston, 1995). However, it is possible to argue a completely opposing case that the increases in PTH with age are a compensatory adaptation that helps to protect against bone loss (Silverberg *et al.* 1996). Whatever the mechanism whereby a suboptimal vitamin D might be seen to exacerbate bone loss, it is at least possible to test practically whether boosting the body stores with vitamin D itself or with an active metabolite has a measurable effect on BMD and/or in preventing fractures. This aspect of vitamin D in bone health is considered next.

## INTERVENTION TRIALS WITH VITAMIN D SUPPLEMENTS

A number of intervention trials have been carried out to see whether the osteoporotic changes in bone with the ageing process can be halted or slowed by improving the vitamin D status of elderly subjects. Such trials have used either vitamin D itself (with or without Ca supplements), or metabolite forms such as the active hormone 1,25-dihydroxy-cholecalciferol or 1  $\alpha$ -hydroxycholecalciferol; the latter being first activated by 25-hydroxylation in the liver. In the sense that supplementation with the metabolically-active form of vitamin D may bypass the metabolic impairment of kidney hydroxylation that may be present in elderly people, 1,25(OH)<sub>2</sub>D treatment may be considered as hormone-replacement therapy rather than a correction of a nutritional deficiency.

*Supplementation with vitamin D: effects on bone mineral density*

The results of several prospective trials using vitamin D supplements (all double-blind, placebo-controlled) have been published in the last 6 years. Most have used daily oral supplements. Usually the main outcome measure has been the change in BMD, although in the larger trials it has been possible to examine the effects on fracture rates. Measurements of 25(OH)D and PTH have provided the biochemical background against which any changes in bone mass could be assessed, and in particular to follow up the hypothesis that secondary hyperparathyroidism caused by a deficit of vitamin D may be a cause of bone loss in the elderly. Summaries of the protocols and main results of the trials with dietary supplements are shown in Table 2.

In Boston, USA, Dawson-Hughes *et al.* (1991) showed that giving 10  $\mu$ g vitamin D daily to post-menopausal women reduced the wintertime bone loss from the lumbar spine and over the 1-year study period produced an overall net benefit in BMD at this site. However, there was no net change between treated and placebo groups in the whole-body BMD. On the basis that the spine is mainly trabecular bone and that the whole skeleton is 80% cortical bone, they suggested that trabecular bone is more sensitive than cortical bone to the effects of vitamin D. The winter and spring levels of 25(OH)D declined from summer and autumn levels by about 25% in the placebo group but by only 5% in the supplemented group. This wintertime fall in 25(OH)D in the placebo group was matched by a significant rise in their wintertime PTH levels that was not observed in the supplemented group. In a follow-up study, the same researchers tried to define more accurately the optimal dose of vitamin D needed to minimize the rate of bone loss in post-menopausal women (Dawson-Hughes *et al.* 1995). This was a 2-year trial in which two groups of post-menopausal women received either 2.5  $\mu$ g or 17.5  $\mu$ g vitamin D as a daily supplement in addition to their usual average dietary consumption of 2.5  $\mu$ g. In contrast to their earlier study the changes in BMD at the spine did not differ between the two groups. However, at the femoral neck, although there was an overall loss in BMD in both groups over the 2-year study, this loss was less in the women given the higher dose of vitamin D. Both groups showed seasonal changes in relative gains during the summer and autumn and losses in winter and spring. In the wintertime, levels of 25(OH)D declined and PTH rose in the lower-vitamin D-intake group but remained stable in the high-intake group. It was concluded that a total daily intake of 5  $\mu$ g vitamin D was insufficient to minimize bone loss from the femoral neck in post-menopausal women living at a latitude of 42°N.

A recent intervention trial from The Netherlands in a more elderly group of post-menopausal women has also indicated the positive benefits of vitamin D supplementation (Ooms *et al.* 1995). After 2 years the vitamin D-supplemented women showed an increase in BMD at the femoral neck but not at the femoral trochanter and distal radius. Since the

Table 2. Randomized, controlled trials with dietary vitamin D supplements

Reference	Study group	n*	Age (years)		Regimen	Duration (years)	Results
			Mean	SD			
Dawson-Hughes <i>et al.</i> (1991)	Healthy, post-menopausal women living independently	249	62	0.5	10 µg vitamin D + 400 mg Ca	1.0	Reduced late wintertime bone loss from vertebrae Net spine BMD† No change in whole-body BMD
Chapuy <i>et al.</i> (1992)	Healthy, elderly women living in nursing homes or in apartments for the elderly	3270	84	6	20 µg vitamin D + 1200 mg Ca	1.5	Hip fractures 43% ↓ Non-vertebral fractures 32% ↓ In subset (n 56), BMD of proximal femur 2.7% ↑ in vitamin D group and 4.6% ↓ in placebo group
Chapuy <i>et al.</i> (1994)†						3.0	Hip fractures 29% ↓ Non-vertebral fractures 24% ↓
Dawson-Hughes <i>et al.</i> (1995)	Healthy post-menopausal women living independently	261	64	5	2.5 µg or 17.5 µg vitamin D + 500 mg Ca	2.0	Loss of BMD from femoral neck lower in 17.5 µg group (-1.06%) than in 2.5 µg group (-2.54%). No difference in BMD at spine
Lips <i>et al.</i> (1996)	Healthy elderly living independently, in nursing homes, or in apartments for the elderly	2578 (1916 women, 662 men)	80	6	10 µg vitamin D	3.5	No difference in fracture incidence. In subset (n 248) of women from nursing homes, BMD 2.3% ↑ after 2 years

↑, Increase; ↓, decrease.

\* No. of subjects enrolled in the study.

† Same study as Chapuy *et al.* (1992) after further 1.5 years of treatment.



latter sites comprise predominantly trabecular bone, this study does not accord with the original suggestion of Dawson-Hughes *et al.* (1991) that trabecular bone is more sensitive to the effects of vitamin D than cortical bone. The increase in BMD at the femoral neck was most apparent after the first year of vitamin D supplementation and, as in the studies from the USA (Dawson-Hughes *et al.* 1991, 1995), was accompanied by significantly increased serum 25(OH)D and significantly reduced serum PTH.

#### *Supplementation with vitamin D: effects on fracture rates*

Vitamin D supplementation trials from France (Chapuy *et al.* 1992, 1994) and The Netherlands (Lips *et al.* 1996) that have been large enough to include fracture incidence as a clinical end-point have shown different results. Thus, whereas the French study (Chapuy *et al.* 1992, 1994) showed significant decreases in both hip fractures and in total non-vertebral fractures after 1.5 and 3 years, the Dutch study (Lips *et al.* 1996) showed no difference in fracture rates after 3.5 years (Table 2).

What factors could account for these differences? The French subjects received a higher dose of vitamin D and extra Ca, their average age was higher and all lived in nursing homes (Chapuy *et al.* 1992, 1994). In contrast about 40% of the Dutch cohort were living independently and 25% were men (Lips *et al.* 1996). All these factors were working in a direction that might be expected to enhance the effect of vitamin D in the French study (Chapuy *et al.* 1992, 1994) and to decrease it in the Dutch study (Lips *et al.* 1996). The benefits in the French study (Chapuy *et al.* 1992, 1994) were supported by densitometric measurements made in a subset of fifty-six women showing a 2.7% increase in BMD of the total proximal femur in the supplemented group compared with a 4.6% decline in the placebo group. This was mirrored by a 44% decrease and 12% increase in serum PTH in the supplemented and placebo groups respectively. These results support the hypothesis that suppression of PTH secretion by correction of a pre-existing vitamin D deficiency may be the chief mechanism underlying the beneficial effect of vitamin D supplementation on BMD in the elderly. However, it does not answer the question as to what degree the decrease in fracture incidence may have been mediated by an increase in bone density or by an improved neuromuscular function leading to fewer falls. As in the French study (Chapuy *et al.* 1992, 1994), a sub-study in 248 women from the Dutch trial (Lips *et al.* 1996) showed that vitamin D supplementation prevented bone loss; after 2 years active treatment, the BMD at the left and right femoral necks had increased by an average of 2.3% (Ooms *et al.* 1995). Again the increased BMD in the Dutch cohort was accompanied by a significant decrease in PTH and, in contrast to the French study (Chapuy *et al.* 1992, 1994), there was a slight but significant increase in 1,25(OH)<sub>2</sub>D. The authors of the Dutch trial (Lips *et al.* 1996) gave two main reasons why the positive effects on BMD might not have been seen or did not translate into fracture benefit. First, the original power of the study would have detected a 20% hip fracture decrease but might have missed the theoretically-predicted 15% decrease in hip-fracture incidence based on the BMD increase actually found. Second, the sub-set on whom BMD measurements were made were all women living in homes for the elderly and were not representative of the larger fracture-study cohort that contained men and persons living independently.

Instead of daily oral supplements of vitamin D, one prospective trial in Finland investigated the effect of giving a single large dose annually by intramuscular injection for 2–5 years (Heikinheimo *et al.* 1992). Analysis of all fractures showed significantly fewer in the treated group but, somewhat surprisingly, this reduction only attained statistical significance for fractures of the upper limb with no discernible protection against hip

fractures. As expected, the fracture rate was much greater in women than men and perhaps because of the small number of male fractures, the effect of vitamin D on fracture reduction was only seen in women.

In conclusion, the trials conducted to date suggest that vitamin D supplements may be beneficial in reducing fracture rates in the elderly, particularly in women living in residential care in northern latitudes. Whether a reduction in fracture rate was shown or not, the mechanical plausibility of a vitamin D effect is supported by the amelioration in bone loss and the biochemical plausibility by increases in serum 25(OH)D and decreases in PTH. For oral supplementation, the question of the optimal dose is still unclear. If the average dietary intake is 2.5 µg/d an additional amount of 2.5 µg/d is clearly too low (Dawson-Hughes *et al.* 1995), but any difference between 10 and 20 µg/d is arguable (Lips *et al.* 1996). The role of Ca supplements is also uncertain and needs to be addressed (Compston, 1995).

#### *Supplementation with vitamin D metabolites*

To date only two vitamin D metabolites 1,25(OH)<sub>2</sub>D (calcitriol) and 1α(OH)D (alphacalcidol) have been used in the treatment of post-menopausal osteoporosis. In Europe and the USA nearly all the trials have used calcitriol, whereas alphacalcidol has been widely used in Japan.

The most reproducible effect of calcitriol is in stimulating intestinal Ca absorption (Reid, 1996). There have been several controlled trials testing the effects on BMD but few large enough or of appropriate design to test the effects on fracture rates. Some of these trials have reported an increase in BMD but some have reported no benefit (for review, see Fujita, 1992, 1996; Reid, 1996). Closer examination of the results suggests that higher doses of calcitriol are more effective with a threshold beneficial dose of about 0.5 µg/d (Fujita, 1992; Bikle, 1994; Dawson-Hughes, 1996). In studies showing a positive effect on BMD, there is also an indication that the greatest benefit is seen at the spine rather than the hip or radius (Dawson-Hughes, 1996). The effect of calcitriol in reducing fracture rates needs to be confirmed because many of the studies have inherent weaknesses in design (Dawson-Hughes, 1996; Reid, 1996).

The controlled trials with alphacalcidol, mostly carried out in Japan, have shown more consistent benefits on bone mass and/or fracture than calcitriol (Reid, 1996). It seems unlikely that this is an inherent property of this compound because its hepatic conversion to 1,25(OH)<sub>2</sub>D takes place rapidly after its administration. It is more likely that the apparent greater effectiveness of alphacalcidol reflects either a dose effect or its use in mainly Japanese women who have low Ca intakes. Thus, the main benefit of either calcitriol or alphacalcidol may be to promote Ca absorption in populations in whom the availability of dietary Ca is a limiting factor to bone growth (Fujita, 1992; Reid, 1996).

The medical concerns of the use of calcitriol or alphacalcidol stem from the associated risk of hypercalcaemia, hypercalciuria, and possibly renal calcification (Fujita, 1992; Reid, 1996). Therefore, the balance of achieving an increase in BMD without unwanted side effects may depend on maintaining a delicate balance of the metabolite dose and dietary intake of Ca (Fujita, 1992, 1996).

In summary, the available evidence suggests that vitamin D metabolites may be useful in the treatment of osteoporosis, but that there is a narrow safety margin between benefit and toxicity (Bikle, 1994; Dawson-Hughes, 1996). It is also an area of substantial controversy. The potential therapeutic benefits need to be considered with specific reference to the individuals or population group in whom their use is proposed, and in the

knowledge that osteoporosis is a heterogeneous condition with a variety of pathogenic origins (Reid, 1996). Ideally the proper use of vitamin D metabolites requires an understanding of their mechanism of action. It is usually assumed that the beneficial effects of vitamin D metabolites accrue from their well-established effects in promoting intestinal Ca absorption but this is far from certain (Bikle, 1994; Dawson-Hughes, 1996). It should be remembered too that even if a specific defect (e.g. Ca absorption) is corrected, this will not necessarily result in a benefit on bone density (Reid, 1996). Again, if the effectiveness of these agents lies in the correction of an underlying vitamin D-deficient state, as is consistently found in very elderly and frail women, then it is safer and cheaper to treat them with physiological doses of vitamin D (calciferol) than their metabolites (Reid, 1996).

#### VITAMIN K: SOURCES OF VITAMIN K

##### *Dietary sources*

The major dietary source of vitamin K is the plant form phylloquinone. Although phylloquinone is widely distributed in foods, green vegetables contain the highest concentrations (Booth *et al.* 1995a; Shearer *et al.* 1996) and represent the single largest contributor to dietary intakes (Booth *et al.* 1996). In general, the relative phylloquinone content of vegetables reflects its association with photosynthetic tissues, with the highest values (3–6 mg/kg) being found in dark-green, leafy vegetables such as kale (*Brassica oleracea acephala*), parsley (*Petroselinum crispum*), spinach (*Spinacia oleracea*) and cabbage and intermediate amounts (1–2 mg/kg) in broccoli, Brussels sprouts (*Brassica oleracea gemmifera*) and lettuce. Certain vegetable oils (e.g. soyabean, rapeseed and olive oils) are also relatively rich sources (0.5–2 mg/kg) and make significant contributions to the diet in margarines and composite foods or when otherwise used for culinary purposes.

The contribution of long-chain menaquinones (MK) to the human diet seems to be limited to a few fermented foods such as cheeses and mainly limited to two major forms MK-8 and MK-9 at concentrations of 50–100 and 100–200 µg/kg respectively; these are thought to derive from the bacterial starter fermentation, because moulds do not synthesize MK (M. J. Shearer and A. Bach, unpublished results). The only animal food products containing nutritionally significant amounts of long-chain MK are livers of ruminant species that may contain MK-7–13 at concentrations in the range of 100–200 µg/kg. However, long-chain MK are very lipophilic and are likely to be poorly absorbed. MK-4 is atypical of the MK series because it is not a common bacterial form but may be present in animal tissues as a result of its synthesis from menadione, or as recent evidence suggests (Thijssen & Drittij-Reijnders, 1994), from phylloquinone itself. The nutritional significance of MK-4 still needs to be evaluated because of the widespread use of menadione salts as an animal feed additive that may enhance the natural MK-4 content of tissues. Some animal tissues and eggs may contain more MK-4 than phylloquinone.

##### *Bacterial synthesis of vitamin K by human intestinal flora*

Many bacteria present in the human intestine synthesize MK and quantitative measurements of their concentrations at different sites have been made by Conly & Stein (1992). The majority of this MK content is present in the distal colon, with MK-10 predominating. However, there is no direct evidence that this potentially large pool of approximately 1 mg colonic MK is bioavailable. Evidence against significant colonic absorption is that the highly lipophilic MK are tightly bound to the cytoplasmic membrane and in the absence of bile salts the only perceivable mechanism of absorption would be via

the portal route (Shearer *et al.* 1996). Experiments in rats have shown that whereas some colonic MK-4 may be absorbed via the portal pathway, there is no detectable transfer of long-chain forms such as MK-9 (Ichihashi *et al.* 1992). On the other hand, the widespread presence in the human liver of very-long-chain forms such as MK-10–13, often at higher concentrations than phylloquinone, does require an explanation. It seems likely that MK-10–13 are of intestinal origin, because they have not been detected in commonly-eaten foods but are typical of the anaerobic genus *Bacteroides* that are major inhabitants of the intestinal tract. The most likely site of absorption would be the terminal ileum where there are still both reasonable concentrations of MK (Conly & Stein, 1992) and bile salts.

#### ACTIONS AND METABOLISM OF VITAMIN K

The only unequivocal biochemical role for vitamin K is as a cofactor for a unique post-translational chemical modification confined to a small group of specialized Ca-binding proteins (Furie & Furie, 1990). The nature of this modification was first shown in 1974 for the hepatic synthesis of prothrombin (factor II) and soon extended to the other classical vitamin K-dependent coagulation factors VII, IX and X. The vitamin K-dependent step is a carboxylation reaction that transforms selective glutamic (Glu) residues in the protein precursor into  $\gamma$ -carboxyglutamic acid (Gla) residues. The reaction is catalysed by a microsomal enzyme called  $\gamma$ -glutamyl or vitamin K-dependent carboxylase (Furie & Furie, 1990) and requires molecular oxygen and CO<sub>2</sub>. The form of vitamin K required as the cofactor is not the stable quinone structure found in the diet, but the reduced quinol (or hydroquinone) structure (KH<sub>2</sub>). The subsequent oxidation of vitamin KH<sub>2</sub> to vitamin K 2,3-epoxide provides the energy for the abstraction of a proton from the  $\gamma$ -carbon of the Glu residue to generate a carbanion that then undergoes carboxylation to yield the final Gla product (Dowd *et al.* 1995). Within the transformed proteins, the Gla residues confer Ca-binding properties and where their function is known (e.g. coagulation proteins) Gla is essential to their biological activity (Furie & Furie, 1990).

In contrast to vitamin D, no metabolic transformations are thought to be necessary for the post-translational carboxylation role of vitamin K, although the relative importance of phylloquinone and MK *in vivo* is poorly understood (Suttie, 1995). There are several findings that suggest that long-chain MK (and by implication floral synthesis) are less important than phylloquinone in meeting human requirements. First, even though most of the liver stores comprise long-chain MK, dietary restriction of phylloquinone alone (which would not be expected to alter floral MK availability) can lead to a sub-clinical under-carboxylation of the hepatic coagulation factors (Suttie, 1995). Second, total liver concentrations of MK are much less labile than phylloquinone to low dietary intakes (Usui *et al.* 1990). This suggests that much of the liver content of long-chain MK might not be readily available to the glutamyl carboxylase. The importance of dietary phylloquinone to the carboxylation status of osteocalcin is suggested by recent findings that show that circulating levels of the under-carboxylated fraction of osteocalcin (ucOC) are responsive to changes in dietary intakes of phylloquinone (Sokoll *et al.* 1997).

Little is known about the metabolism of vitamin K in bone. There is one published study of the vitamin K content of human cortical and trabecular bone which showed the presence of phylloquinone and three MK congeners (MK-6, MK-7 and MK-8) with phylloquinone generally predominating (Hodges *et al.* 1993b). Preliminary results from our laboratory have confirmed that phylloquinone is the major form in human bone but the MK pattern was different (M. J. Shearer, N. Kakkad and S. J. Hodges, unpublished results). We found only MK-4, -5, -6 and -7 in bone lipid; their concentration decreased with the side-chain length and no higher homologue than MK-7 was detected. This pattern is strikingly

different from the well-established MK spectrum of the liver in which very-long-chain MK (e.g. MK-10–13) usually predominate.

The putative presence of MK-4 in bone is intriguing, because a series of studies from Japan suggest that this congener may have a direct role in inhibiting bone resorption through a different mechanism than  $\gamma$ -carboxylation. This MK homologue, known as menatetranone in Japan, has been shown to inhibit bone loss in rats, an effect that may be mediated by both the suppression of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) synthesis (Hara *et al.* 1993) and the inhibition of osteoclast-like multinucleated cell formation (Akiyama *et al.* 1994). Further studies by the same group suggested that this inhibitory effect is specific to the geranylgeranyl side chain of menatetranone and is even displayed by the free alcohol, geranylgeraniol (Hara *et al.* 1995). More recently, Kameda *et al.* (1996) presented evidence that MK-4 may inhibit osteoclastic bone resorption by targeting osteoclasts to undergo apoptosis.

#### *Vitamin K-dependent bone proteins*

Compared with the well-established role of vitamin D in bone health, an appreciation that vitamin K also plays a role in skeletal metabolism came comparatively recently, with the discovery in the mid 1970s that mineralized tissue contained a large reservoir of Gla and that this was associated with at least one major protein (Hauschka *et al.* 1975; Price *et al.* 1976). This protein became known as osteocalcin or bone Gla protein (BGP) and accounts for up to 15 % of the non-collagenous bone of vertebrate species (Robey & Boskey, 1996).

Besides osteocalcin, two other vitamin K-dependent proteins have been isolated from bone tissue: matrix Gla protein (MGP) and protein S (Robey & Boskey, 1996). Neither protein, however, is exclusive to bone tissue. MGP is also found in cartilage and is expressed by a variety of other soft tissues, while protein S was already known to have an important endothelial function in the feedback control of the coagulation cascade. The roles of MGP and protein S in bone metabolism are uncertain (Binkley & Suttie, 1995; Vermeer *et al.* 1995). However, the original suggestion of Price (1985) that MGP may inhibit cartilage mineralization has been strongly supported by the recent discovery that gene-targeted mice that lack MGP undergo spontaneous calcification of soft tissues; one consequence of this is inappropriate calcification of various cartilages including the growth plate, leading to short stature, osteopenia and fractures (Luo *et al.* 1997). With respect to the role of vitamin K, it seems likely that the teratogenic effect of warfarin in causing excessive fetal calcification is at least partly due to the inhibition of MGP function by this vitamin K antagonist (Binkley & Suttie, 1995). These ill-effects of vitamin K antagonists on bone health seem to be largely confined to rapidly-growing bone. The possible effects of vitamin K antagonists on adult bone function are controversial, some studies reporting a reduced BMD and others reporting no effect (Binkley & Suttie, 1995).

#### *Osteocalcin, a vitamin K-dependent bone protein modulated by vitamin D*

Osteocalcin is a small protein, typically forty-nine or fifty residues, with three Gla residues situated at residues 17, 21 and 24 (Price, 1985). Although there is no homology between osteocalcin and the classical vitamin K-dependent coagulation factors, there has been a high degree of conservation of osteocalcin itself throughout evolution. For example, the position of the three Gla residues in bovine and human osteocalcin are exactly the same as in the swordfish (*Xiphias gladius*) which diverged from terrestrial vertebrates some 400 million years previously (Price, 1985).



An important physico-chemical property that the three Gla residues confer on osteocalcin is a high adsorption affinity to hydroxyapatite. This affinity to hydroxyapatite is far stronger than that to  $\text{Ca}^{2+}$  and seems to be due to the way in which the three Gla residues project outwards from the Gla helix, and their spacing arrangement that complements the interatomic spacing of the  $\text{Ca}^{2+}$  of the hydroxyapatite lattice (Hauschka & Carr, 1982). It has recently been demonstrated that osteocalcin isolated from twenty different human bone specimens is incompletely carboxylated and that the degree of  $\gamma$ -carboxylation of Glu residues is strongly dependent on their sequence position; the mean values for the percentage  $\gamma$ -carboxylation at each of the three residues being 67% at residue 17, 88% at residue 21 and 93% at residue 24 (Cairns & Price, 1994). The correlation between the degree of  $\gamma$ -carboxylation at the two most sensitive glutamate residues of 17 and 21 suggested that the incomplete carboxylation observed in each individual occurred during post-translational modification, possibly due to an insufficiency of vitamin K in bone cells. Of the adult cohort, although most were elderly (average age 79 years), the incomplete carboxylation reflects the picture in bone matrix synthesized over several years rather than at the time of sampling.

The synthesis of osteocalcin by osteoblasts is modulated by, but not absolutely dependent on,  $1,25(\text{OH})_2\text{D}$  (Price, 1985). This stimulating effect on osteocalcin synthesis was first observed *in vitro* in an osteosarcoma cell line (Price & Baukol, 1980) and later *in vivo* when the  $1,25(\text{OH})_2\text{D}$  metabolite was shown to result in a 3–4-fold increase in either young or old rats (Price & Baukol, 1981). In human subjects too, there is some evidence that circulating osteocalcin levels may be influenced by  $1,25(\text{OH})_2\text{D}$  but the picture is more complex. Lian & Gundberg (1988) concluded that while cell-culture and animal studies have shown strong correlations between the effects of  $1,25(\text{OH})_2\text{D}$  on serum osteocalcin, clinical studies in human subjects have shown a response but not normally a direct correlation. The measurement of circulating osteocalcin has become in its own right one of the most useful clinical biochemical markers of bone turnover and specifically bone formation (Price, 1985; Lian & Gundberg, 1988).

The function of osteocalcin is unclear. In reviewing the then available evidence, Price (1985) favoured a role as an inhibitor of mineralization. Others, based on early evidence of the chemotactic properties of osteocalcin have favoured a role in skeletal resorption, whereby the protein acts as a matrix signal in the recruitment and differentiation of osteoclasts (Glowacki *et al.* 1991; Liggett *et al.* 1994). Chenu *et al.* (1994) used human osteoclast-like cells from giant cell tumours of bone to demonstrate for the first time that osteocalcin also displays chemotactic activity in a validated human osteoclastic model. They also showed that osteocalcin promoted adhesion and spreading of the osteoclast-like cells followed by the synthesis and release of bone matrix proteins such as osteopontin and bone sialoprotein. Very recently, mutant mice with the osteocalcin gene deleted have been shown to exhibit a phenotype characterized by higher bone mass, with evidence that this was due to an increase in bone formation (Ducy *et al.* 1996). Both mineralization and resorption appeared normal in the mutant mice, but studies in ovariectomized animals showed a more rapid increase in bone resorption in the osteocalcin-deficient animals compared with the wild-type animals. This suggests that in oestrogen-deficient animals the lack of osteocalcin results in a more rapid bone loss than in normal animals (Ducy *et al.* 1996).

#### ASSESSMENT OF VITAMIN K STATUS

In the last 10 years or so considerable advances have been made in the development of methods which can be used to gain insights into the vitamin K status of individuals and



population groups (Shearer, 1995). There have been two main approaches. The first, as with vitamin D, has been to try and assess whether circulating levels of vitamin K can be used as an index of tissue reserves of the vitamin. The second, more functional approach, is to try and assess the extent of  $\gamma$ -carboxylation of a target vitamin K-dependent protein. The rationale behind this approach is that a reduced supply of vitamin K will result in the production of under-carboxylated (des- $\gamma$ -carboxy) species of the Gla protein that, if also released into the plasma, can be assayed. In practice, although there are many vitamin K-dependent proteins in the body, assays of under-carboxylated species are available for only two proteins, i.e. the coagulation protein prothrombin and the bone protein osteocalcin.

#### *Plasma measurements of vitamin K*

Unlike other fat-soluble vitamins, including vitamin D, reliable methods for the analysis of vitamin K in plasma and tissues have become available only comparatively recently and their relative complexity has restricted their wider use (Shearer, 1995). Most assays measure only phylloquinone, the predominant dietary and transport form. The measurement of MK-7 and -8 in plasma has been reported in some studies (Hodges *et al.* 1990, 1991, 1993a).

Vitamin K has no specific binding protein in plasma and is carried in the circulation by lipoproteins. After an overnight fast, more than half the circulating phylloquinone is still associated with triacylglycerol-rich lipoproteins, thought to be of intestinal rather than hepatic origin, with the remainder being equally distributed between LDL and HDL (Kohlmeier *et al.* 1996).

Plasma levels of phylloquinone are sensitive to changes in recent dietary intake and in healthy volunteers fall in response to diets that have been moderately (Suttie *et al.* 1988) or severely (Ferland *et al.* 1993) depleted in vitamin K. When the dietary intake of phylloquinone was severely depleted from 80  $\mu\text{g}/\text{d}$  to about 10  $\mu\text{g}/\text{d}$ , plasma levels of phylloquinone fell dramatically within a few days to about 15% of the initial values (Ferland *et al.* 1993). A similar rapid drop in plasma phylloquinone in response to a low dietary intake (about 5  $\mu\text{g}/\text{d}$ ) was observed in hospitalized patients (Usui *et al.* 1990). A study assessing dietary intakes and plasma phylloquinone in 402 post-menopausal women showed that they were significantly related, although the correlation coefficient ( $r$  0.13,  $P=0.01$ ) was quite low (Booth *et al.* 1995b). Stronger relationships between dietary intakes and plasma phylloquinone were obtained in a recent UK study (C. Bolton-Smith, R. J. G. Price, S. T. Fenton, D. J. Harrington and M. J. Shearer, unpublished results).

#### *Assays of under-carboxylated vitamin K-dependent proteins*

Assays for under-carboxylated prothrombin have been available for many years (Widdershoven *et al.* 1987). However, most lack the sensitivity to detect minor changes in the Gla content of prothrombin. The exception are new generation immunoassays based on monoclonal antibodies to under-carboxylated prothrombin (Widdershoven *et al.* 1987; Shearer, 1995).

Methods for the assessment of ucOC have been less satisfactory because they have usually relied on an indirect method of assessing the Gla content based on the differential affinity of Gla-rich and Gla-depleted osteocalcin for hydroxyapatite (Price & Kaneda, 1987; Knapen *et al.* 1989; Merle & Delmas, 1990) or for  $\text{BaSO}_4$  (Sokoll *et al.* 1995). All these methods suffer from methodological problems that are inherent to osteocalcin assays in general and that relate to the heterogeneity of circulating osteocalcin and its instability in

serum samples (Blumsohn *et al.* 1995). Despite these drawbacks there is evidence that these adsorption methods do give useful information about the carboxylation status of osteocalcin in the circulation that reflects the Gla content of newly-synthesized osteocalcin in the bone. Future studies will be helped by the development of a specific assay for ucOC (Vergnaud *et al.* 1997).

Recent support that ucOC measurements are of value as a marker of vitamin K nutritional status has come from a study in 263 healthy subjects (aged 18–85 years) from the Boston area that has shown that ucOC is positively correlated with under-carboxylated prothrombin and negatively correlated with plasma phylloquinone (Sokoll & Sadowski, 1996). The direction of these associations with two established indicators of vitamin K nutritional status are those that would be expected if ucOC was also an index of vitamin K status.

The value of ucOC measurements as an index of bone vitamin K status has been underlined by recent findings showing that the proportion of circulating ucOC responds rapidly to reasonably modest changes in dietary intakes of phylloquinone (Sokoll *et al.* 1997). Thus, when the daily dietary intakes of young, healthy subjects residing in a metabolic ward were increased from 100 µg to 420 µg the ucOC fraction fell by 41 % after 5 d and then rose again during the next 5 d when they returned to the 100 µg diet. Interestingly, in a control group who were maintained for the same period on the 100 µg diet the percentage of ucOC rose significantly, by 28 %. The plasma phylloquinone levels also reflected the dietary changes. One implication of this study is that a large proportion of the population in both the UK (Price *et al.* 1996) and the USA (Booth *et al.* 1996) is consuming much less than 100 µg/d and that these intakes are insufficient to keep osteocalcin in a fully-carboxylated state.

#### VITAMIN K AND OSTEOPOROSIS

Relatively little is known about the adequacy of the vitamin K status of different age-groups in relation to bone health. Until recently there has been little reason not to believe that most people, including the elderly, have sufficient dietary intakes of vitamin K. This perception has been based mainly on the extreme rarity of a coagulopathy that can be attributed to a nutritional deficiency of vitamin K. However, with the availability of more sensitive assays for both under-carboxylated prothrombin and ucOC it is now possible to make independent assessments of the sufficiency of vitamin K in the tissues (liver and bone) that synthesize these two Gla proteins. This has led to the concept of tissue specific requirements for vitamin K (Vermeer *et al.* 1995).

##### *Vitamin K status of the elderly: dietary intakes*

With the increasing availability of accurate food data bases for phylloquinone (Booth *et al.* 1995a; C. Bolton-Smith, R. J. G. Price, S. T. Fenton, D. J. Harrington and M. J. Shearer, unpublished results) it is now becoming possible to assess dietary intakes in different population groups. Estimations of dietary intakes of phylloquinone in the US Food and Drug Administration (FDA) Total Diet Study across fourteen age–gender groups suggested that intakes for adults were close to the current US recommended dietary allowances (RDA; National Research Council, 1989) of 80 µg for men and 65 µg for women, although intakes were slightly lower in the 25–30 years age-group (Booth *et al.* 1996). The same researchers had earlier reported a much higher average dietary intake of 156 µg in 402 healthy post-menopausal women (Booth *et al.* 1995b). The discrepancy could reflect the

different methods used to estimate dietary intakes or could be a true reflection of the wide range of dietary intakes in different population groups. In the first detailed study of phyloquinone intakes in the UK, sixty-five Scottish volunteers aged 22–54 years had average daily intakes of 64 and 72  $\mu\text{g}$  for men and women respectively (Price *et al.* 1996). This UK study was based on 7 d weighed food records made during spring, summer, autumn and winter months and showed no seasonal variation. However, with an intra-individual CV of 103%, the study showed that there were extreme variations in daily intakes that could easily mask any seasonal trend. The average UK intakes are similar to those reported in the US FDA Total Diet Study (Booth *et al.* 1996) and close to the current adult dietary reference values for the UK of 1  $\mu\text{g}/\text{d}$  per kg body weight (Department of Health, 1991). However, although the average UK intakes were in line with the guidelines, a significant number of the Scottish population were consuming less than the reference intakes judged to be both safe and adequate. As with the US RDA, they are also based on estimates of the needs to maintain coagulation function (about 1  $\mu\text{g}/\text{d}$  per kg body weight) rather than on any precepts of the needs for optimal bone health. Nothing is yet known about dietary intakes of phyloquinone in an elderly UK population.

#### *Vitamin K status of the elderly: plasma vitamin K*

In a study of the factors influencing plasma phyloquinone levels in 326 healthy people from New Mexico, it was observed that there were no sex differences but that young women had significantly lower levels than elderly women (Sadowski *et al.* 1989). However, when the phyloquinone concentrations were expressed relative to triacylglycerol levels, the picture was reversed and the adjusted values were lower in the elderly of both sexes. This adjustment can be justified on the basis of the strong association of circulating phyloquinone with triacylglycerol (Sadowski *et al.* 1989; Kohlmeier *et al.* 1995) and suggests that the elderly may have decreased reserves of phyloquinone. In a small study in the UK, there was no difference in circulating concentrations of phyloquinone or MK-7 between young and elderly healthy individuals, although in the elderly group there appeared to be a deficit of MK-8 (Hodges *et al.* 1990). However, a similar small French study showed no difference between serum phyloquinone, MK-7 or MK-8 in young and elderly women (Plantalech *et al.* 1991). Since the validity of measurements of plasma MK, especially MK-8, remain to be established, the significance of this discrepancy between these two studies is at present uncertain.

As illustrated in Fig. 1, plasma measurements made in osteoporotic patients have consistently shown dramatically reduced levels of phyloquinone, and possibly MK, in patients who had sustained femoral neck or spinal crush fractures (Hart *et al.* 1985; Hodges *et al.* 1991, 1993a). It has been difficult, however, to assess whether this relationship is fortuitous or causal, but it does not seem to be related to trauma (Hodges *et al.* 1993a).

#### *Vitamin K status of the elderly: under-carboxylated osteocalcin*

The measurement of circulating ucOC perhaps represents the most relevant marker of vitamin K with respect to its role in bone health because it provides information on the carboxylation status of a highly abundant vitamin K-dependent protein in bone that is not synthesized in significant amounts by other tissues. The first indication that the elderly tend to have increased levels of ucOC came from a Dutch study comparing osteocalcin measurements in premenopausal (25–40 years) and post-menopausal (55–75 years) women, the latter being recruited from a convent (Knapen *et al.* 1989). Measurements of

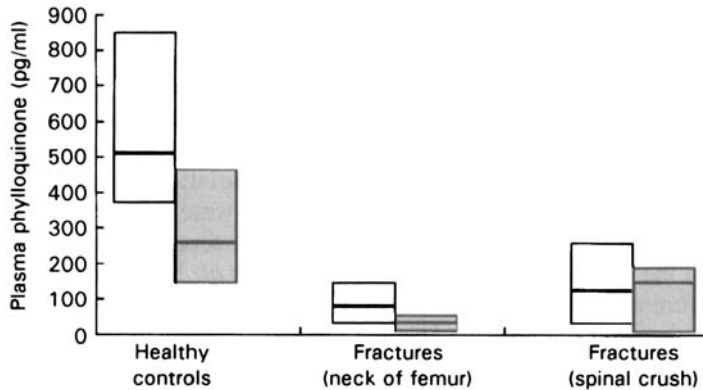


Fig. 1. Circulating levels of phyloquinone (□) and menaquinone-7 (■) in patients with osteoporotic fractures of either the neck of femur or spine. Values shown are interquartile ranges and median values (—). (Data taken from Hodges *et al.* 1991.)

total osteocalcin and the fraction that remained unbound after incubation with hydroxyapatite showed that the percentage of bound osteocalcin (termed by these researchers the 'hydroxyapatite-binding capacity') was more than 50% lower in the postmenopausal women than in the premenopausal group. An age-related impairment in  $\gamma$ -carboxylation of osteocalcin in women was also suggested by a cross-sectional French study in three age-groups in which it was found that levels of ucOC were significantly increased after the menopause and markedly increased in old age (Plantalech *et al.* 1991). This increase in the ucOC fraction was separate from the increase in total antigenic osteocalcin after the menopause that has often been reported and was also seen in the French study of Plantalech *et al.* (1991). The increase in ucOC with age was found whether ucOC was expressed as an absolute concentration or as percentage of total osteocalcin. The very high levels of ucOC seen in the elderly group (in some women ucOC was as high or higher than levels seen in warfarin-treated patients) may have reflected both their particularly great age (average 85 years) and the fact that they were recruited from a retirement home. However, the finding of normal average serum phyloquinone in a subset of the elderly women, while not ruling out a suboptimal vitamin K status, did not suggest a major vitamin K deficiency in this elderly group (Plantalech *et al.* 1991). Other reasons could be an age-related impairment of the transport system of vitamin K to osteoblasts, a defect in the intrinsic osteoblast  $\gamma$ -carboxylation mechanism or some other resistance to the action of vitamin K that may be analogous to the tissue resistance to vitamin D seen in the elderly.

The large French vitamin D-supplementation study of Chapuy *et al.* (1992) has also been the setting of a small osteocalcin-based substudy by Szulc *et al.* (1993) who showed that the relative risk of hip fracture during follow-up was six times higher in institutionalized, elderly women who had an initially raised ucOC value. ucOC was the only biochemical marker in this study that showed any predictive value for hip-fracture risk and was later shown to correlate with hip BMD (Szulc *et al.* 1994). The subsequent findings by the same group that serum ucOC was also predictive of fracture risk in a much larger cohort of women living independently (Vergnaud *et al.* 1997) strengthens the significance of their earlier studies and shows the potential value of ucOC measurements in the assessment of hip-fracture risk. Although vitamin K deficiency seems the most likely cause of a raised ucOC, unexpected findings were the inverse relationship between serum

ucOC and 25(OH)D and the significant lowering of ucOC by vitamin D supplementation (Szulc *et al.* 1993). This remains unexplained but raises the possibility of an influence of vitamin D on vitamin K-dependent carboxylation through an unknown mechanism.

#### *Effects of vitamin K supplementation on biomarkers of bone metabolism*

Part of the Dutch study demonstrating the age-related changes in the hydroxyapatite-binding capacity of osteocalcin (Knapen *et al.* 1989) consisted of a supplementation study with a daily dose of 1 mg phylloquinone for 2 weeks. While phylloquinone had no effect on osteocalcin in young women, the proportion of ucOC in post-menopausal women rose substantially to near normal values. There was also a rise in serum levels of total immunoreactive osteocalcin in the post-menopausal group. Moreover, in a subset of about 20% of the post-menopausal women, classified as 'fast losers' of Ca with evidence of increased bone turnover, there was a substantial and significant decrease in both urinary Ca and hydroxyproline excretion. The effect of 1 mg phylloquinone/d in decreasing urinary Ca excretion in post-menopausal women with initially high Ca output was seen in a second study (Knapen *et al.* 1993).

In Japan, a large intervention trial in osteoporotic women has been carried out with large doses (45 mg/d) of MK-4 (menatetranone) with encouraging results, as judged from measurements of BMD and markers of bone turnover. The detailed results of this study carried out by Orimo and co-workers are available only in abstract form in English language journals but have been discussed in the reviews of Binkley & Suttie (1995) and Vermeer *et al.* (1995).

#### CONCLUSIONS

In the last few years there has been growing evidence that a suboptimal status of vitamin D or vitamin K may contribute to the pathogenesis of osteoporosis. To date the evidence of a beneficial effect of these vitamins is much stronger for vitamin D for which the results of intervention trials with supplements have been encouraging. There are also plausible mechanisms to explain both the origins and ill effects of a suboptimal vitamin D status, and the benefits of supplementation to bone mass. Comparable compelling evidence of the beneficial effects of vitamin K to osteoporosis is currently lacking. The potential relevance is based on findings of biochemical indicators of an impaired vitamin K status in osteoporotic patients or to associations of markers of vitamin K status and those of bone turnover. At the nutritional and biochemical level there is now good evidence that the dietary intakes of vitamin K needed to sustain maximal carboxylation of bone Gla protein such as osteocalcin are substantially higher than those needed for carboxylation of the coagulation Gla proteins synthesized in the liver (Vermeer *et al.* 1995; Sokoll *et al.* 1997). This knowledge together with clinical evidence, such as the increased fracture risk associated with a raised ucOC, is already compelling enough to warrant intervention studies with vitamin K of similar designs to those already carried out with vitamin D. Thus far, the only vitamin K supplementation studies carried out have been in Japan with pharmacological doses of MK-4, a form that is not a major dietary component but for which there is nevertheless evidence of a tissue specific distribution and of having anti-resorptive properties in bone. The field is still hampered by the lack of knowledge of the physiological function of the vitamin K-dependent proteins in bone and of other possible mechanisms whereby vitamin K can improve bone mass and/or quality.



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