Vitamin D and its metabolites in the pathogenesis and treatment of osteoporosis

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Summary

Vitamin D and calcium are essential for normal skeletal growth and for maintaining the mechanical and structural integrity of the skeleton. Reduced intake of calcium and vitamin D may be associated with reduced bone mass and osteoporosis while a chronic and severe vitamin D deficiency may lead to osteomalacia. 1,25$(OH)_2$D (calcitriol) is the major active metabolite of vitamin D and promotes intestinal calcium absorption and the mineralization of bone matrix and reduces PTH secretion. Despite vitamin D and calcium are considered essential components of management strategies for the prevention and treatment of osteoporosis, many people do not have adequate vitamin D levels. Vitamin D insufficiency is particularly common in the elderly due to reduced exposition to sunlight, declined synthesis of vitamin D in the skin and impaired renal hydroxylation. Even though secure inferences from randomized controlled trials on the prevention of osteoporotic fracture with vitamin D or its metabolites are limited, these compounds have been demonstrated to be pharmacologically active, safe and cost-effective for the prevention of age-related bone loss. Their use should be encouraged expecially in elderly subjects or in condition of dietary deficiencies. Interestingly, health benefits of vitamin D and its analogues may go beyond osteoporosis, including prevention of cancer and autoimmune diseases and improvement of neuromuscular function.

KEY WORDS: vitamin D, calcitriol, alfacalcidiol, osteoporosis, fracture.

Introduction

Osteoporosis is the most prevalent metabolic bone disease among developed countries and it is defined as a skeletal disorder characterized by compromised bone strength and increased risk of fracture (1). Its clinical significance lies in the occurrence of fractures, involving most commonly the forearm, the vertebral bodies and the hip, but fractures at other sites may be also associated with the disease. For Caucasians, the lifetime risk of an osteoporotic fracture at 50 years of age has estimated to be approximately 40% for women and 13% for men (2). Each year more than 1.5 million people suffer hip, vertebral, and wrist fractures due to osteoporosis, a disease that can be prevented and treated. The occurrence of osteoporotic fractures leads to considerable mortality, morbidity, reduced mobility and decreased quality of life (3). Actually, the annual number of hip fractures in 15 countries of European Community (EC) has been estimated to be 500,000, with a total care cost of about 4.8 billion euros per year (4). This burden will increase in absolute terms because of the ageing of the population. Given the magnitude of the problem, the prevention and treatment of osteoporosis is, therefore, of major importance for health organizations in all countries.

The major determinant of bone strength and osteoporotic fracture risk is bone mineral density (BMD), as assessed by dual photon absorptiometry or dual energy x-ray absorptiometry. According to WHO criteria, osteoporosis is defined to exist when BMD values fall more than 2.5 standard deviations below the young adult reference mean (1). Many studies indicated that the risk of fragility fractures increases progressively as BMD declines (5-8). It has been estimated that the risk of new vertebral fractures increases by a factor of 2.0-2.4 for each standard deviation decrease in BMD, irrespective of the site of bone density measurement (8). However, bone strength depends not only on BMD. Bone size as well as bone quality are other important components that interact with BMD in determining the risk of fracture (1).

Skeletal bone mass is determined by a combination of endogenous (genetic, hormonal) and exogenous (nutritional, physical activity) factors. In adolescence to attain the optimal peak bone mass the main determinants are genetic, nutritional and behavioural (exercise). In adult and elderly populations the main determinants of age-related bone loss are represented by the gonadal status, by the influence of some nutrients and by the physical activity. Both a low peak bone mass and a high rate of bone loss represent risk factors for osteoporosis and osteoporotic fractures. Nutrition plays an important role in bone health. The two nutrients essential for bone health are calcium and vitamin D, even though an adequate nutritional intake of nutrients and a regular exercise are also necessary (9, 10). Vitamin D and calcium are important for normal skeletal growth and for maintaining the mechanical and structural integrity of the skeleton. Reduced supplies of calcium may be associated with a reduced bone mass and osteoporosis, whereas a chronic and severe vitamin D deficiency leads to osteomalacia, a metabolic bone disease characterized by a decreased mineralization of bone matrix and an increased osteoid volume. Vitamin D deficiency can be confirmed by measuring the serum concentration of 25-hydroxy-vitamin D [25(OH)D], which is the major circulating metabolite and represents the storage form of vitamin D. In patients with osteomalacia, serum 25(OH)D levels are usually below 5-6 ng/ml and often undetectable (10-12). Biochemically osteomalacia is characterized by normal or low serum concentrations of calcium and phosphate, and increased activity of alkaline phosphatase. Vitamin D deficiency is common in the elderly, particularly in institutionalised subjects. The major causes of vitamin D deficiency are a scarce exposition to sunlight, a decline in the synthesis of vitamin D in the skin, a poor nutrition, and a decreased renal hy-

droxylation of vitamin D (12). A subclinical vitamin D deficiency, is also common with ageing. This condition, that has been defined vitamin D insufficiency (10, 11), is increasingly being recognized as a distinct pathological skeletal entity, characterized by normocalcemia and normal bone mineralization and by an increase in circulating levels of parathyroid hormone (PTH). In the presence of osteoporosis, vitamin D insufficiency may amplify bone loss and thus enhance fracture risk. It follows that at any age, but particularly in postmenopause and in the elderly, an adequate intake of both calcium and vitamin D, is important for the preservation of bone mass and prevention of osteoporosis (10, 13, 14).

Vitamin D and bone homeostasis

Vitamin D is important for bone, for its essential role in promoting intestinal calcium absorption and mineralization of bone matrix. The major source of vitamin D is the skin, where it is produced by the action of ultraviolet light on steroid precursors. Vitamin D is also present in a limited number of foods, and the dietary sources of the vitamin can be important under circumstances of decreased sunlight exposure. Dietary vitamin D is absorbed in the small intestine via the intestinal lymphatic system in the presence of bile acids. Vitamin D is derived from plant (vitamin D_2 or ergocalciferol) and animal sources (vitamin D_3 or cholecalciferol). The main dietary sources of vitamin D are fatty fish (salmon, sardines, tuna) and oils derived from them, some meat products (liver), eggs and wild mushrooms. Vitamin D (D_3 and D_2 collectively) is not a true vitamin, but a pro-steroid hormone that is biologically inert until metabolised. It is transported to the liver bound to a specific α-globulin (vitamin D binding protein), and to a small extent albumin and lipoproteins. In the liver, vitamin D is metabolised to 25(OH)D, which functions as the major storage form by virtue of its long half-life. In the kidney 25(OH)D (25-hydroxy-cholecalciferol) is further metabolised by a 1α-hydroxylase enzyme to 1,25$(OH)_2$D (calcitriol), the hormone responsible for the biological effects of vitamin D. Metabolism of vitamin D may be even more complex, since recent studies demonstrated the existence of extrarenal 1α-hydroxylases in many tissues, including bone and muscle (15). Locally produced 1,25$(OH)_2$D may therefore act as a paracrine/autocrine factor. The binding of calcitriol to the vitamin D receptor (VDR), a nuclear steroid hormone receptor, activates VDR to interact with retinoid X receptor (RXR) and form the VDR/RXR/co-factor complex, which binds to vitamin D response elements in the promoter region of target genes to regulate gene transcription (16). These receptor are located in classical target tissues including bone, intestine, kidney and parathyroid glands as well as in many other tissues or cell types such as skin, muscle, and the immune system (16, 17). Although the classical genomic pathway is responsible of most biological activity of vitamin D, some effects may be mediated by cell surface receptors through non-genomic pathways (18, 19).
Calcitriol is the major biologically active metabolite of vitamin D. The principal regulators of 1,25$(OH)_2$D production are PTH, 1,25$(OH)_2$D itself, dietary intake of calcium and phosphate. There are three primary target organs for circulating 1,25$(OH)_2$D: intestine, parathyroid gland and bone. In the intestine, calcitriol induces the expression of an epithelial calcium channel, calcium-binding protein (calbindin), and a variety of other proteins to help the transport of dietary calcium into the circulation (20). In the skeleton, calcitriol influences bone remodeling, mainly by acting on osteoblasts, in which it stimulates the production of osteocalcin and different cytokines (21-23). VDRs have been identified in osteoblasts and in several osteoblastic cell lines while their presence in osteoclasts is still under debate (24). In the parathyroid glands, 1,25$(OH)_2$D markedly decreases PTH gene transcription and parathyroid cell proliferation and induces parathyroid cell differentiation (25, 26). Thus, the overall effects of 1,25$(OH)_2$D on mineral metabolism may be summarized as: 1) increased intestinal calcium absorption, leading to increase in serum calcium; 2) decreased of serum PTH level (both through direct inhibition of PTH secretion from the parathyroid gland and indirect inhibition of PTH secretion by the raised serum calcium levels); 3) decreased bone resorption (mainly due to a reduction in the PTH-mediated bone resorption); and 4) under certain conditions increased bone formation (27). Usually, under normal vitamin D status, the small intestine absorbs about 30% of dietary calcium. In the absence of calcitriol, intestinal calcium absorption is solely by the passive, extracellular route, which limits gross calcium absorption to about 10-15% of intake.
The vitamin D status may be evaluated by measuring the serum concentration of 25(OH)D, the main circulating form of vitamin D. Since 25(OH)D concentrations are mainly affected by sunlight exposure and intensity (28), the two major determinants of serum 25(OH)D levels are the season and the geographical location. In fact, the cutaneous synthesis of previtamin D is maximal in summer and minimal in winter (Fig. 1), and an inverse association exists between mean 25(OH)D concentration and latitudes. There is no general consensus on normal serum 25(OH)D values (29). Clearly, reference levels of vitamin D will depend on the age, skin pigmentation, season, latitude and sun exposure of the population studied. Moreover, variation among different assays and among different laboratories using the same assay have been described (30, 31). Several studies have been performed to establish a threshold of normal 25(OH)D levels in different populations based on an increased risk of secondary hyperparathyroidism, low BMD or high bone turnover. Even though these threshold estimates varied among different studies there is common opinion that in healthy adult subjects the optimal serum 25(OH)D level for bone health is approximately 30 ng/ml (75 nmol/l) (11, 32). When serum 25(OH)D values fall below this threshold, there is a progressive increase in PTH secretion (11, 33) that may increase bone resorption. Moreover, it has been estimated that 25(OH)D levels below 32 ng/ml (80 nmol/l) are associated with decreased calcium absorption (33). A long-lasting and severe deficiency of vitamin D, as defined by a serum level of 25(OH)D lower than 6 ng/ml (15 nmol/l), is associated with defective mineralization resulting in rickets in children and osteomalacia in adults. Vitamin D insufficiency, the preclinical phase of vita-

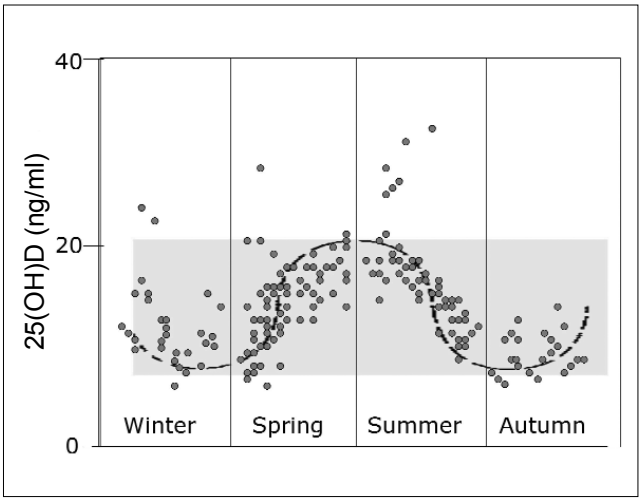

Figure 1 - Annual variations of 25-(OH) serum levels in healthy subjects with age range between 66 and 95 years (adapted from Lorè F et al., Italian Journal of Mineral and Electrolyte Metabolism. 1988;2:93-106).

min D deficiency, as defined by a serum level of 25(OH)D comprised between 6 and 30 ng/ml, causes a reduced calcium supply and a secondary hyperparathyroidism. If this state remains chronic, osteopenia results. In addition to its role in calcium homeostasis several data suggested a direct effect of vitamin D on muscle strength and low serum 25(OH)D levels have been also associated with neuromuscular consequences, including lower extremity muscle weakness and increased risk of falling (34, 35).

Calcium, vitamin D and osteoporosis

Calcium and vitamin D are recognized as essential nutrients throughout life for skeletal growth and maintenance of bone mass. Calcium is the prevalent mineral of bone and its absorption from the intestine depends on vitamin D. Convincing evidence has been given that dietary calcium intake is positively related to BMD in children and adolescents (36). In adolescent, the higher the calcium intake, the greater the peak bone mass (37). A positive correlation between bone mass and calcium intake has been demonstrated also in adult (38, 39). Although there are many factors which modulate the progression of age-related bone loss syndromes, the pathogenesis of this process has been attributed, at least in part, to decreased calcium absorption by an "aging intestine", to an associated elevation in circulating PTH, and to decreased synthesis of $1,25(OH)_2D$ (13, 40). Decreased $1,25(OH)_2D$ synthesis by the aging kidney results from both age-related progressive loss in the capacity of the renal 1-α-hydroxylase to respond to progressive elevation in PTH and an age-related decrease in the circulating 25(OH)D precursor (40). Women with osteoporosis have been often demonstrated to be characterized by reduced intestinal calcium absorption when compared with age-matched control subjects (39, 41). This abnormality is particularly relevant in those women who have a low calcium diet. Moreover, increments in serum osteocalcin levels observed in humans treated with $1,25(OH)_2D$, and animal studies demonstrating increased number of bone marrow osteoblast precursors during $1,25(OH)_2D$ administration are consistent with the hypothesis that abnormalities in the production and skeletal distribution of $1,25(OH)_2D$ may also contribute to the defects in osteoblast function and bone formation (42, 43). Dietary calcium and vitamin D intake as well as solar exposure generally decreases with increasing age, and the dermal production of vitamin D following a standard exposure to UVB light can be impaired in elderly subjects, due to atrophic skin changes (29, 44). Moreover, several epidemiological studies, performed in different populations, demonstrated that a low vitamin D status is common among adult (11, 13, 45, 46) and elderly (47-49) populations, regardless of latitude. All these changes in vitamin D metabolism render the ageing population at higher risk of vitamin D deficiency, especially in winter seasons and may lead to severe consequences in terms of fall, osteoporosis and fractures (Fig. 2). Importantly, both type I (postmenopausal) and type II (senile) osteoporosis appear to be, at least in part, related to primary and secondary abnormalities of vitamin D-endocrine system (50). Similar abnormalities have been also described in corticosteroid-induced osteoporosis, the most common cause of secondary osteoporosis (51). Recently, the observation that VDR genotypes may be predictive of differences in bone mass and intestinal calcium absorption in postmenopausal women adds further complexity to this issue (52-54). Some subjects may be in fact genetically predisposed to impaired calcium homeostasis and could be particularly sensitive to reduction in calcium intake and/or vitamin D levels.

In the last decade, a positive association between serum 25(OH)D concentrations and bone mass has been reported in adult and elderly populations from North America and Europe. Several studies have shown a relationship between BMD, vitamin D insufficiency and secondary hyperparathyroidism in the elderly (55, 56) but also in postmenopausal and middle-aged women (57, 58). In a cross-sectional study conducted in the UK a positive relation between serum 25(OH)D values and BMD was observed in a group of middle-aged women (58). In an American study performed in postmenopausal women with low vertebral bone mass, a relation between vertebral BMD and PTH values was found only in subjects with low 25(OH)D values (57). In another study a significant association between low femoral BMD and low 25(OH)D levels was described in normal women older than 60 years (55). A more recent study in 13,432 men and women from the NHANES III cohort (age > 20 yrs), serum 25(OH)D and total hip BMD were positively associated up to 25(OH)D levels of 90 to 100 nmol/l (59). An increased risk of fracture with low levels of vitamin D and reduced risk with vitamin D supplementation has been observed in many but not all studies (10, 13). It has been estimated that individuals with 25(OH)D levels below 68 nmol/L have a 4 time increased fracture risk over 8 years, independently of BMD. This risk further increases, reaching 19 times in patients with osteoporosis (60). Furthermore, women with acute hip fracture showed markedly reduced bone levels of $1,25(OH)_2D$ as well as reduced circulating 25(OH)D levels (Fig. 3) with respect to controls in different studies (61-63).

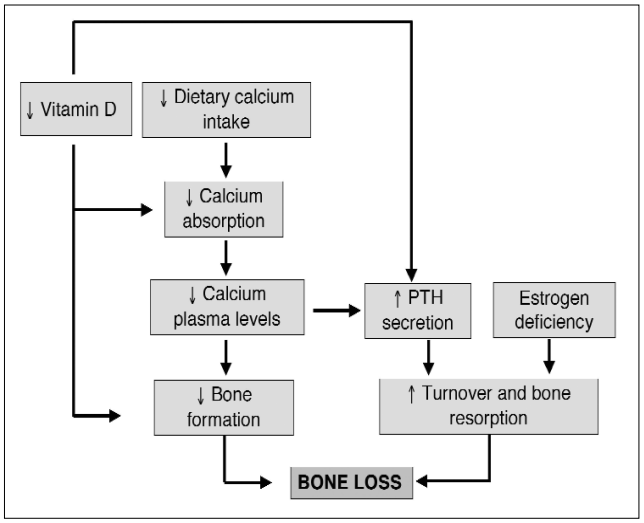

Figure 2 - Schematic representation of the relationships between calcium, vitamin D endocrine system and bone loss.

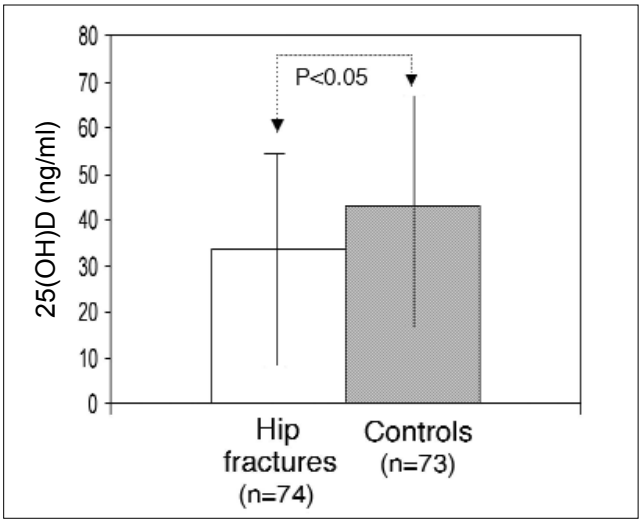

Figure 3 - 25(OH)D levels in women with and without acute hip fractures (adapted from Nuti et al., Clin Orthop. 2004;422:208-213).

Vitamin D and its metabolites in the treatment of osteoporosis

Relatively small changes in vitamin D status may have significant effects on bone mass. This justifies the use of vitamin D and its active metabolites in the prevention and treatment of osteoporosis, since vitamin D deficiency or insufficiency has been found to be frequent in the elderly as well as in postmenopausal women, particularly during low sun exposure.

Treatment with vitamin D

The most rational approach to reducing vitamin D insufficiency is supplementation. It has been estimated that the body uses on average 3,000-5,000 IU daily of cholecalciferol (64). Even though both vitamin D_2 and D_3 can be used, ergocalciferol is only about 20-40% as effective as cholecalciferol in maintaining serum 25(OH)D concentrations, since is more rapidly catabolized (65). Fortification of foods with vitamin D provides an alternative approach to direct supplementation. This approach is common in the US, where milk is fortified with vitamin D, and less common in Europe with the exception of some Northern countries (Belgium, Netherlands and United Kingdom) where fortification is compulsory only to margarines (66). In Europe, the recommended dietary allowances (RDA) for vitamin D have been proposed in 1998 by the report on osteoporosis-action on prevention (66). The requirement for dietary vitamin D, based on European and Nordic recommendations, depends on the amount of sunshine exposure, with an higher limit estimated for individuals with minimal endogenous synthesis and a lower limit for individuals able to produce adequate vitamin D. However, a common RDAs of 400 IU (10 mg) daily has been proposed for people aged 65 years or over (66). Even though a similar RDA of 400 IU irrespective of age is also established by the US Food and Drug Administration, the proposed Institute of Medicine Adequate Intake (AI) for the US and Canada is 400 IU daily for subjects 70 years or younger, and 600 IU (15 mg) daily for those over age 70 years (67-70). Importantly a daily intake of 600 IU of vitamin D_3 is needed to reach a mean serum 25(OH)D level of 50 nmol/l and at least 800-1000 IU (20-25 mg) is needed to attain a mean level of 75 nmol/l, that is close to the suggested threshold for optimal skeletal health (32). Thus, according to these recent evidences, higher vitamin D intakes than those currently recommended by RDAs and AI should be advised, especially in the elderly or other high-risk populations. As counterpart, excess vitamin D intake should be also avoided. Although the maximum safe dose is still unknown and doses of 4000 IU daily have been given without toxicity (71, 72), intakes of 50 mg (2000 IU) daily should not be exceeded to avoid some harm full effects, such as hypercalcemia and hypercalciuria (73, 74).

Some randomised controlled prospective studies and recent meta-analyses confirmed the role of calcium and vitamin D supplementation in the prevention of bone loss and osteoporosis, even though they differed in baseline calcium and vitamin D status, in age range or in study design (75, 76). Importantly, as concluded by a recent Cochrane review (76) the relative contribution of vitamin D and calcium to these benefits was less clear. Although longitudinal data have suggested a role of vitamin D intake in modulating bone loss in peri- and postmenopausal women, most of the studies of vitamin D and calcium supplementation have failed to support a significant effect of vitamin D and calcium during early menopause. In a 2 year double-blind study on early postmenopausal women (mean age 57 yrs), whose average baseline serum 25(OH)D concentration was well within the normal range, the addition of 10,000 IU vitamin D weekly to calcium supplementation at 1000 mg/day did not confer benefits on BMD beyond those achieved with calcium supplementation alone (77). Similarly, in a randomized, co-twin, placebo-controlled, double-blind study in early postmenopausal twin pairs, vitamin D supplementation (800 IU/day) as a single agent for 2 years was not effective on BMD and bone markers (78). In contrast, a more recent double-blind, randomised, 30-month controlled trial in 120 peri- (age range 45-50 yrs) and early (age range 50-55 yrs) postmenopausal women showed a positive effect of calcium and vitamin D supplementation (Ca 500 mg and vitamin D 200 UI, daily) on BMD change with respect to placebo (79). In another study in healthy postmenopausal women and men (mean age 57.9±11 yrs) supplementation with oral vitamin D (500 IU) and calcium during winter abolished seasonal changes in calciotropic hormones and markers of bone turnover, leading to an increase in BMD with respect to placebo (80). There is a clearer skeletal benefit in vitamin D and calcium supplementation in older postmenopausal women. Vitamin D intake between 500 and 800 IU daily, with or without calcium supplementation, has been shown to increase BMD in Caucasian women with a mean age of approximately 60-70 years. Conversely, in a study in calcium-replete postmenopausal African American women there was no observed effect of vitamin D_3 supplementation (800 IU/day) for 2 years on bone loss or bone turnover markers (81). In Caucasian women older than 65 as well as in elderly men, there is even more benefit. This latter point has been clearly addressed by a number of studies on the effects of vitamin D supplementation on bone loss in the elderly. Some studies have been performed with vitamin D alone, some others with vitamin D and calcium supplementation (82-84). It appears from these studies that supplementations with daily doses of 400-800 IU of vitamin D, given alone or in combination with calcium (1200-1500 mg daily), are able to reverse vitamin D insufficiency, to reduce secondary hyperparathyroidism, to prevent bone loss and to improve bone density in elderly subjects (10, 13, 73). Beneficial effects on BMD seems greater at the femoral neck than lumbar spine or forearm (84). Of interest, a positive and significant effect on BMD has been observed within the first year of treatment in a randomized, double-blind, placebo-controlled study on elderly women with vitamin D deficiency, defined as serum 25(OH)D concentrations 12 ng/ml (85). Moreover, both vitamin D alone (vitamin D_2 300,000 IU single injection or oral vitamin D_3 800 IU/day) and vitamin D associated with calcium resulted in a small but significant improvement in BMD, suppressed parathyroid hormone, and reduced falls as compared to controls in a recent secondary prevention study (86).

Even though most studies documented a positive effect on BMD, the role of vitamin D supplementation on fracture risk is still controversial (Fig. 4). In a retrospective epidemiological case-control study conducted in six European Mediterranean countries (MEDOS study) treatment with vitamin D was reported to be associated with a slight but not significant reduction in the incidence of hip fracture (87). Randomized controlled trials showed conflicting results. A systematic meta-analysis indicated that a clear reduction in hip (pooled RR 0.74, 95% CI 0.61-0.88) and non-vertebral (pooled RR 0.77, 95% CI 0.68-0.87) fracture risk of vitamin D at doses of 700-800 IU/day, but not at 400 IU/day (88). However, the relative effect of vitamin D alone or vitamin D plus calcium was not investigated. A more recent meta-analysis (89) showed no statistically significant effect of vitamin D alone on hip fracture (seven trials, 18,668 participants, RR 1.17, 95% CI 0.98 to 1.41), vertebral fracture (four trials, 5698 participants, RR – random effects 1.13, 95% CI 0.50 to 2.55) or any new fracture (eight trials, 18,903 participants, RR 0.99, 95% CI 0.91 to 1.09). Conversely, vitamin D with calcium marginally reduced hip fractures (seven trials, 10,376 partici-

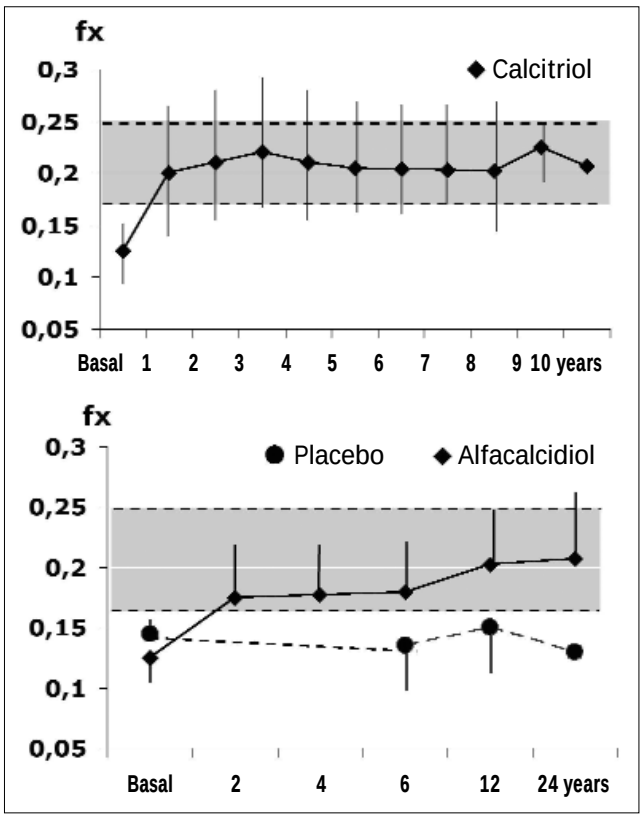

Figure 4 - Relative risk with 95% interval of confidence of vertebral fractures after treatment with standard vitamin D compounds (with and without calcium supplementation). Major randomized, double blind controlled studies have been considered.

pants, RR 0.81, 95% CI 0.68 to 0.96), non-vertebral fractures (seven trials, 10,376 participants, RR 0.87, 95% CI 0.78 to 0.97), but there was no evidence of effect of vitamin D with calcium on vertebral fractures. The effect appeared to be restricted to those living in institutional care. The first prospective, large, multicenter, randomised, double-blind, placebo controlled study was performed on a French cohort of over 3000 institutionalised elderly women (mean age 84 years) during treatment with either vitamin D (800 IU/day) and calcium (1.2 g/day) or placebo for three years (90). Active treatment significantly reduced the incidence of new hip fractures by 29% and that of all non vertebral fractures by 24% (90). Similar results on hip fracture prevention (RR 1.6; 95% CI 0.96-3.0) were obtained in a multicenter, randomised, double-masked, placebo-controlled confirmatory study on 583 French ambulatory institutionalised women (mean age 85.2 years) (91) as well as in a placebo-controlled trial of the effect of calcium (500 mg/day) and vitamin D (700 IU/day) in healthy community-based men and women older that 65 years (82). After three years 12.9% of subjects treated with placebo and 5.9% of those treated with vitamin D and calcium sustained non-vertebral fractures, a statistically significant difference (82). Moreover, two different studies showed that vitamin D_3 treatment by itself at doses of 100,000 IU every 4 months (92) or 30,000 IU annually (93) reduces the occurrence of fractures in elderly subjects. Lower vitamin D doses showed no significant reduction in fracture rate (94, 95). In a Dutch study, the administration of 400 IU daily of vitamin D to elderly subject with a high calcium intake produced no reduction in fracture rate (94). Similarly, intervention with 400 IU daily of vitamin D_3 failed to prevent either hip or vertebral fractures over a 2-year people in a nursing home population (95). Despite these positive results, recent reports from three large clinical trials, raised major concerns about the role of vitamin D supplementation on fracture prevention (96-98). The first was a randomized placebo-controlled trial performed in 5,292 elderly people (70 years or older) who had had a low trauma fracture before being enrolled (96). Treatment with calcium (1000 mg/day) and vitamin D_3 (800 IU/day), either alone or in combination, for at least 24 months was not effective in secondary prevention of further fractures with respect to placebo. A second study on a larger sample of 36,282 postmenopausal women enrolled in a Women's Health Initiative clinical trial recently analyzed the role of long term (average follow-up period 7 yrs) calcium (1000 mg/day) and vitamin D_3 (400 IU/day) supplementation for primary prevention of hip and other fractures (97). Despite a small but significant increase in total hip BMD, no significant reduction in hip fracture risk was observed in calcium and vitamin D group with respect to placebo (12% reduction, hazard ratio 0.88, 95% confidence intervals 0.72-1.08). Similarly no significant reductions in clinical vertebral, wrist or total fractures were reported. Moreover, there was a small but significant increase in kidney stones in women treated with calcium and vitamin D with respect to placebo (hazard ratio 1.17, 95% confidence intervals 1.02-1.34), that appeared to be unrelated to high baseline calcium intake. It is conceivable that the effect of calcium and vitamin D supplementation on fracture reduction might require higher doses of vitamin D than the 400 IU used in this study. Adherence to treatment might also be considered. Of interest, 41% of women didn't take the intended dose and in secondary subgroup analysis, the preventive effect on hip fracture was greater and significant in women who adhered to the treatment regimen (29% reduction, hazard ratio 0.71, 95% confidence intervals 0.52-0.97) as well as in women over 60 years of age and women not taking personal calcium supplements (97). In the third study, higher doses of vitamin D (800 IU/day) with calcium (1000 mg/day) were given for 18-42 months to 3,314 elderly women with one or more risk factors for hip fracture (98). After a median follow-up of 25 months the occurrence of fracture was lower than expected but not significantly differed with respect to placebo.

Finally, although the possible effects of calcium and vitamin D in fracture prevention are generally attributed to increases in BMD, it has also been hypothesized that supplementation might increase muscular strength, thereby reducing the risk of falls (34, 35). In a study involving a group of 122 elderly institutionalised women, 50% of whom had vitamin D insufficiency, musculoskeletal function was shown to improve significantly) after 3 months' therapy with calcium and vitamin D, while there was no significant improvement in women treated with calcium alone (99). The incidence of falls in the women who received calcium and vitamin D was reduced by 49%. Likewise, Pfeifer and colleagues observed a reduction in body sway and a reduction in falls with short-term calcium and vitamin D supplementation as compared with calcium alone in a sample of 148 elderly women (100).

Taken all together, these studies underline the need for an adequate vitamin D and calcium nutrition in the elderly, particularly in individuals living indoors in nursing homes, who have a high prevalence of vitamin D deficiency or insufficiency.

Treatment with active vitamin D metabolites

The 1α-hydroxylated forms of vitamin D, 1,25-dihydroxycholecalciferol or calcitriol, and 1α-hydroxy-cholecalciferol or alfacalcidiol, have been proposed as possible therapies for osteoporosis (101, 102). Both compounds strongly stimulate the intestinal calcium absorption, and the response is dose-dependent (Fig. 5). This leads to a suppression of PTH secretion and a decrease in bone turnover. Over the past two decades, several clinical trials have been performed in osteoporotic patients

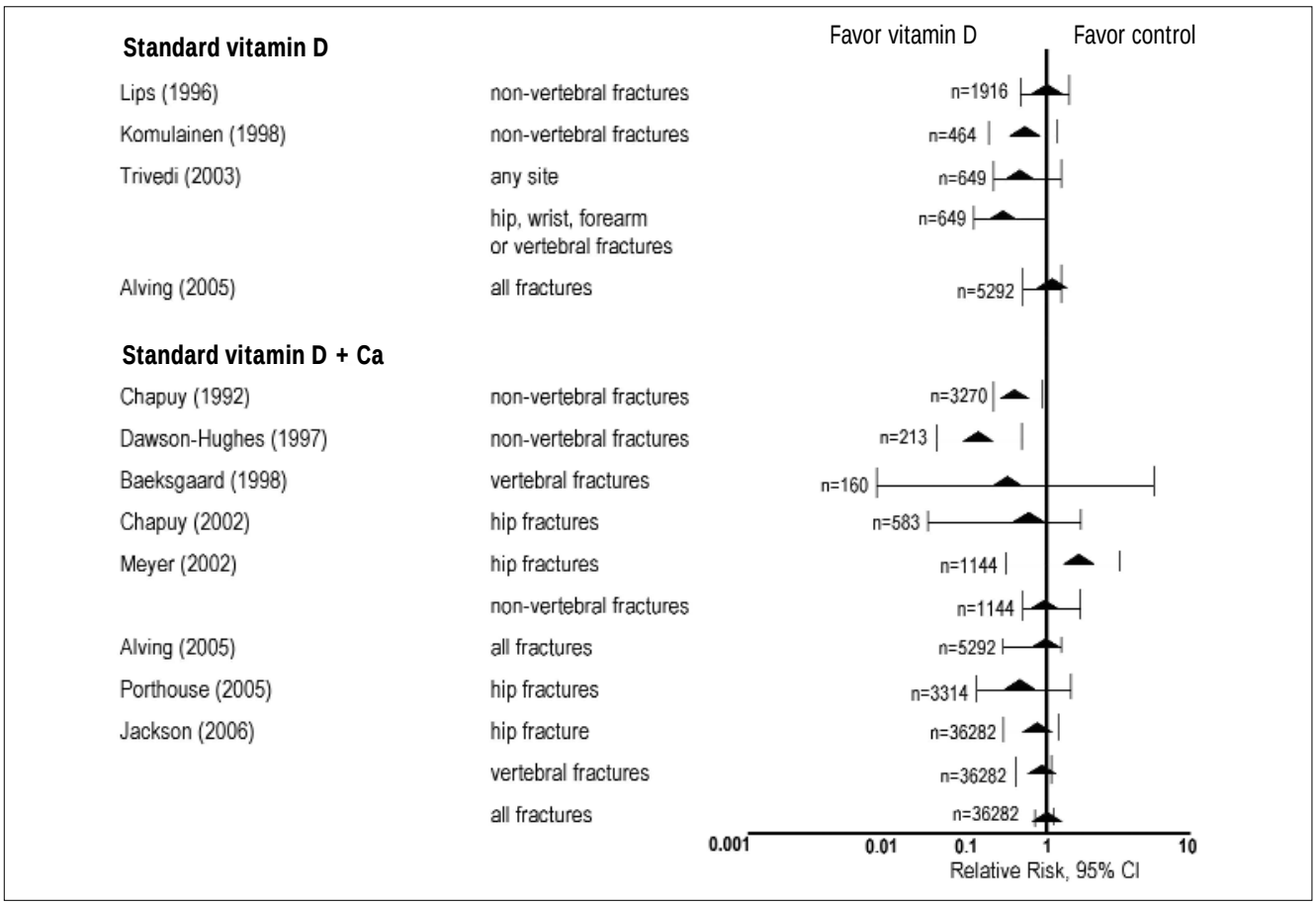

Figure 5 - Fractional radiocalcium absorption (fx) during long term treatment with calcitriol or alfacalcidiol in postmenopausal osteoporotic patients. Dotted lines indicate the normal range (adapted from Caniggia A et al., Osteoporosis Int. 1993;1:S181-S185).

using calcitriol or alfacalcidiol, at doses from 0.25 to 2.0 µg/day. A positive effect of both compounds on BMD was seen in some clinical trials, whereas in others there was no change in BMD (13, 50, 103). In addition, there is not yet a definite answer as to whether these compounds decrease the incidence of osteoporotic fractures. A meta-analysis on published randomised controlled trials showed a consistent, statistically significant effect of hydroxilated vitamin D compounds in all BMD sites for doses above 0.43 mcg, with an overall effect that was higher than that of standard vitamin D treatment (75). A more recent meta-analysis revised the overall effect of active vitamin D metabolites (alfacalcidiol and calcitriol) on BMD and fracture rate (104). A global effect in preventing bone loss in patients not exposed to corticosteroids was demonstrated. Moreover, active vitamin D metabolites significantly reduced the overall fracture rate (RR 0.52, 95% CI 0.46-0.59) and both vertebral (RR 0.53, 95% CI 0.47-0.60) and non-vertebral (RR 0.34, 95% CI 0.16-0.71) fracture rate, respectively (104). The therapeutic effects seem to be pharmacological rather than physiological, and some concern exists about the potential side effects of this treatment. Hypercalcemia and impairment of renal function are rare with lower doses (up to 0.5 µg/day) but more frequent with higher doses (1-2 µg/day). For these reasons, treatment with calcitriol or alfacalcidiol necessitates monitoring of serum calcium and renal function, unlike treatment with vitamin D.

A number of clinical trials have been conducted over the past few years with the aim of verifying the efficacy of calcitriol in postmenopausal and involutional osteoporosis (105-113). Due to differences in studies design a dosage used these studies led to conflicting results, with bone mass and vertebral fracture rate reported as improving in some studies but remaining unchanged or worsening in others. One of the most likely explanations for the negative results is that these studies used ineffective doses, because it seems that the efficacy of 1 α-hydroxylated vitamin D_3 compounds is dose-related. It is now more widely appreciated that the window of efficacy for calcitriol is quite narrow (101-103). Whereas 0.4 µg/day may be insufficient for increasing calcium absorption in many patients, a dose of 0.5 µg/day or higher is effective in nearly all patients with an appropriate supply of dietary calcium (not higher than 0.8 µg/day so as not to increase to the risk of hypercalcemia). Using dosages ranging from 0.5 to 0.8 µg/day, calcitriol has been shown to increase total body and spine BMD in studies on postmenopausal women (103). However, there are few data yet on the effect of calcitriol on fracture incidence (Fig. 6). A significant effect on vertebral fracture rate was obtained in one study (105), while another showed a positive but not significant trend (106). In the former study, 0.5 µg calcitriol per day decreased the incidence of vertebral fractures in women with postmenopausal osteoporosis and with fewer than five fractured vertebrae, but not in those with more than five fractured vertebrae (106). A more recent double blind randomised trial investigated the effect of calcitriol or oestrogen therapy on BMD and the incidence of falls and fractures (107). A reduced incidence of falls and non vertebral fractures over a 3 years period was demonstrated in the calcitriol and oestrogen plus calcitriol groups with respect to placebo.

In addition to its role in postmenopausal and involutional osteo-

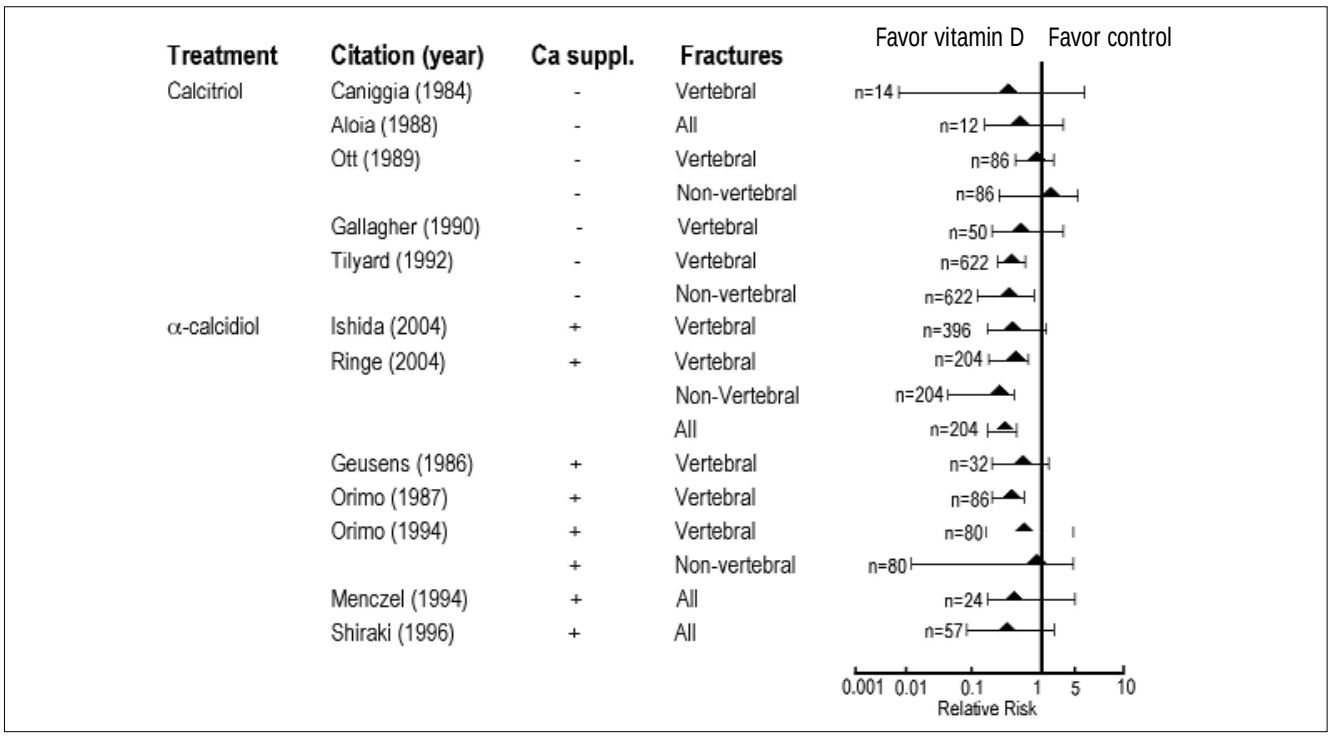

Figure 6 - Relative risk with 95% interval of confidence of vertebral fractures after treatment with active vitamin D metabolites (calcitriol or calcidiol with and without calcium supplementation). Major randomized, double blind controlled studies have been considered.

porosis, calcitriol was used in the treatment of secondary osteoporosis, especially corticosteroid-induced osteoporosis (104, 114, 115) and post-transplantation bone loss (116-120). Corticosteroids result in impaired gastrointestinal absorption of calcium and increased urinary calcium loss leading to secondary hyperparathyroidism with enhanced bone resorption, as well as having direct inhibitory effects on osteoblasts and bone formation. However, even though vitamin D analogs were found to be active in preventing hip and spinal bone loss in corticosteroid induced osteoporosis, their effect appear lower than that observed with bisphosphonates (115) and further data are required regarding fracture prevention in patients receiving glucocorticoid therapy (121). The mechanism of action of calcitriol in preventing transplant osteoporosis may be related to effects on either corticosteroid or cyclosporine pathways, especially secondary hyperparathyroidism, or to its immunomodulatory properties with consequent immunosuppressive agent sparing (116). Recent clinical trials also suggest an important future role of calcitriol as adjunctive therapy to bisphosphonates and estrogen in the treatment of osteoporosis. In particular, a significant benefit of calcitriol treatment combined with HRT on BMD at different skeletal sites has been demonstrated in postmenopausal women (109, 122).

Alfacalcidiol is a synthetic precursor of $1,25(OH)_2D_3$ that has to be converted into $1,25(OH)_2D_3$ predominantly by vitamin D 25-hydroxylase before to exert its biological effects. This activation takes places even with advanced liver disease. Because of these pharmacokinetic aspects, alfacalcidiol is characterized by a more safe profile with respect to calcidiol as regard the risk of developing hypercalcemia or hypercalciuria. This active vitamin D analogue is a pharmacologically active compound, which acts independently of vitamin D status (101, 102, 104, 123). Alfacalcidiol was initially synthesized in order to treat the bone disease of patients with chronic renal disease more effectively since the renal 1α-hydroxylation of $25(OH)D_3$ was compromised in these individuals (124). Subsequently, observations showed that osteoblasts contain a 25-hydroxylase enzyme which converts $1\alpha OHD_3$ into $1,25(OH)_2D_3$, and that there are higher skeletal concentrations of $1,25(OH)_2D_3$ after $1\alpha OHD_3$ administration than after $1,25(OH)_2D_3$ despite the fact that serum levels of $1,25(OH)_2D_3$ were lower after $1\alpha OHD_3$ than after $1,25(OH)_2D_3$ administration (123). Thus, a small proportion of administered alfacalcidiol may be directly activated in bone by a 25-hydroxylase expressed by osteoblasts, suggesting a localised autocrine or paracrine effect in bone tissue, in addition to the systemic effects. In vivo and in vitro experimental evidences suggested that alfacalcidiol not only normalizes PTH and suppresses bone resorption but also simultaneously stimulates bone formation (125). Alfacalcidiol was demonstrated to improve intestinal calcium absorption in osteoporotic women and has been utilized to treat osteoporosis for more than 20 years in Japan (126-128). In different studies, this

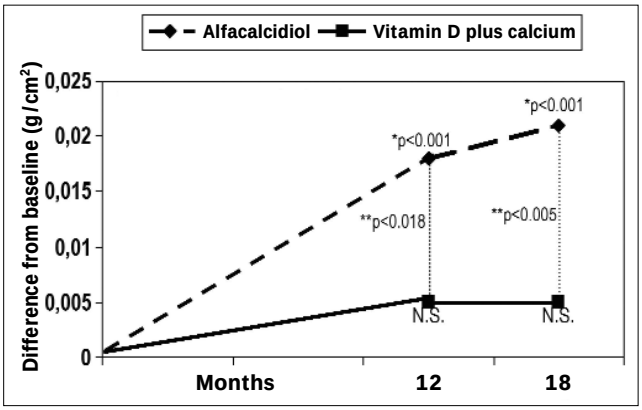

Figure 7 - Lumbar BMD changes from baseline during treatment with alfacalcidiol or vitamin D plus calcium (adapted from Nuti et al., Rheumatol Int. 2006;26:445-453).

compound was demonstrated to maintain BMD, and prevent the occurrence of osteoporotic fractures. Positive effects on bone turnover and lumbar (Fig. 7), femoral neck, or radial BMD have been reported with alfacalcidiol doses ranging from 0.5 to 1.0 µg in both early and late postmenopausal women (129-132). There is now some evidence that alfacalcidiol may be particularly active in conditions characterized by an increased rate of bone loss (133, 134). Alfacalcidiol 1 µg/day fully prevented vertebral bone loss over 3 years in women after the first year of menopause (133). Moreover, improvement in bone turnover, increase in BMD, and reduction in fracture rates have been described during alfacalcidiol treatment in corticosteroid-induced osteoporosis (135, 136), in inflammation-induced bone loss (137, 138), in patients following end organ transplant (139) and in patients with chronic renal failure (140). Importantly, recent clinical comparative studies suggested an increased efficacy of alfacalcidiol over vitamin D in the treatment of postmenopausal (132) and glucocorticoid induced osteoporosis (141, 142). Compared to plain vitamin D, alfacalcidiol exerts higher bone-protective effects, thus allowing the doses to be minimized and lowering the risk of adverse effects, including hypercalcemia. Notwithstanding these observations, the anti-fracture efficacy of this compound remains currently doubtful, since variable results for bone loss and fractures reduction have been demonstrated in different trials varying according to their designs, methodologies, and outcomes (Fig. 6). In particular, bone loss and fracture prevention at the hip level, while being the most invalidating outcome of osteoporosis, remain under-investigated in long-term randomized controlled trials (104). Moreover, its efficacy with respect to other treatment regimens in osteoporosis (i.e. bisphosphonates) also remains to be fully elucidated. While a study in postmenopausal women indicated that switching to 1α-hydroxycholecalciferol therapy after short-term HRT permitted maintenance of increase in bone mass due to HRT for at least 18 months (143), other studies demonstrated a lower efficacy of alfacalcidiol with respect to bisphosphonates or calcitonin (144, 145). Conversely, several studies demonstrated that alfacalcidiol may be an excellent partner for combination therapy (i.e. with HRT) to improve the antifracture efficacy, especially in elderly patients (146-150).

Among the multiple pleiotropic effects of alfacalcidiol its positive action on muscle strength and neuromuscular coordination may exert an important and additional role in fracture risk reduction (151). Treatment with alfacalcidiol in elderly people significantly decreased the high risk of falls (152, 153). Moreover, the effect of alfacalcidiol in increasing the number and diameter of type 2 fibre muscles was demonstrated in elderly women with osteoporosis, after 3 months of treatment (154).

Recently, vitamin D analogs have been developed, that retain the suppressive action on PTH and parathyroid gland growth, but that have less calcemic and phosphatemic activity. Currently, two analogs, 19-nor-1,25-$(OH)_2D_2$ and 1α(OH)D_2, are being used for the treatment of secondary hyperparathyroidism in the United States, and two are being used in Japan, 22-oxacalcitriol and 1,25-$(OH)_2$-26,27F6D_3 (155). Elucidation of the mechanism of action for the different vitamin D analogs in the next future will enhance our understanding of the vitamin D pathway and improve their therapeutic uses in osteoporosis.

Conclusions

Vitamin D and calcium are important for normal skeletal growth and for maintaining the mechanical and structural integrity of the skeleton. Even though secure inferences from randomized controlled trials on the prevention of osteoporotic fracture with calcium and vitamin D supplementation are very limited, expecially in women within the first years of menopause, these compounds have been demonstrated to be pharmacologically active, safe, and cost-effective for the prevention and treatment of osteoporosis. Their use should therefore be encouraged, particularly in the elderly as well as in other conditions of dietary deficiences. Moreover, over the last 30 years, several clinical trials have reported the efficacy of vitamin D-hormone analogs, calcitriol and alfacalcidiol, as possible additional therapies for osteoporosis. In recent comparative studies as well as in meta-analyses these compounds showed an overall increased skeletal efficacy compared to plain vitamin D (104, 121, 132, 141, 142). However, their role in fracture prevention, as well as their relative efficacy with respect to other treatment regimens in osteoporosis (i.e. bisphosphonates), remain under-investigated in long-term randomized controlled trials. Certainly, there is consistent and increasing evidence that treatment with vitamin D analogs or calcium plus vitamin D supplementation exert a synergistic effect with antiresorptive agents on bone, and, thus, most patients will derive further benefit in terms of fracture prevention from the addition of an antiresorptive agent.

Further studies will be needed to evaluate the relative impact of different vitamin D formulations as well as the relative contribution of calcium and vitamin D compounds on fracture prevention. Interestingly, health effects of vitamin D and its analogues may go beyond osteoporosis including prevention of cancer, autoimmune diseases and diabetes, or improvement of neuromuscular function (34, 35, 156-158).

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