

■ COVER STORY: TRI-POINT SERIES

From The Endocrine Society's Research Affairs Committee
Co-edited by Steven Grinspoon, M.D., and Ellen Seely, M.D.

3 PERSPECTIVES on Vitamin D's Role AS A HORMONE

Seeing a Hormone in a Vitamin

Vitamin D has long been regarded as a vitamin. In fact, it has been called the antirachitic vitamin, because conditions caused by its deficiency—such as rickets in children and osteomalacia in adults—can be treated with natural foodstuffs like fatty fishes and fish liver oils. It is, however, more accurately a hormone. Cholecalciferol, vitamin D₃, the so-called sunshine vitamin, is synthesized in the skin from 7-dehydrocholesterol upon exposure to solar UVB. A common synthetic form of vitamin D, which has the structure of ergocalciferol, is vitamin D₂. Both forms are properly regarded as pro-hormones, because activation is necessary, first by C²⁵-hydroxylation in the liver and then by C¹-hydroxylation in the kidneys, and because they are thereafter carried in the blood to target tissues.





Giving the basic scientist's point of view is Julie Glowacki, Ph.D., of Brigham and Women's Hospital, Boston, Mass.



Giving the clinical scientist's point of view is Bess Dawson-Hughes, M.D., of Tufts University, Boston, Mass.



Giving the clinical practitioner's point of view is Michael McClung, M.D., of the Oregon Osteoporosis Center, Portland, Ore.

From the Basic Scientist:

Vitamin D, Calcium, and Bone Mineralization

Vitamin D deficiency is common in the United States and worldwide.¹ In the adult, chronic vitamin D deficiency is associated with osteomalacia, the inadequate mineralization of bone matrix that leads to bone pain, radiographic evidence of pathognomonic pseudofractures, and histological evidence of accumulation of unmineralized osteoid matrix, as well as back pain and muscle weakness. Vitamin D deficiency is also associated with secondary hyperparathyroidism and osteoporosis.

The activated form of the hormone, dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$], is best known as a calcemic agent, regulating calcium and phosphate transport. Its most pronounced effect is to increase synthesis of the calcium-binding protein calbindin in the intestine and the kidney, the two major target tissues involved in calcium homeostasis. By promoting calcium absorption in the intestine, vitamin D ensures sufficient calcium for vital functions and prevents resorption of the mineral from skeletal stores. Modern diets are relatively low in calcium and aging diminishes its intestinal absorption. Thus, elders need greater supplementation than younger adults of both calcium and vitamin D.

Vitamin D Status and Morbidity

Studies show increased prevalence of vitamin D insufficiency in patients with fragility fractures; in one study, half of community-dwelling hip fracture patients had vitamin D deficiency, and more than a third had secondary hyperparathyroidism.² Muscle pain and diminished muscle strength are hallmarks of vitamin D deficiency in adults. Recent epidemiological data on the associations of vitamin D deficiency with arthritis and with non-skeletal diseases, such as colon, prostate, and breast cancers, type 1 diabetes, multiple sclerosis, cardiovascular disease, hypertension, and possibly type 2 diabetes and schizophrenia, raise new questions about direct and indirect effects of the hormone.³ Detection of the vitamin D receptor (VDR) and/or synthesis of $1,25(\text{OH})_2\text{D}$ in many tissues led to studies of possible pathophysiological mechanisms for those associations. For example, $1,25(\text{OH})_2\text{D}$ is a potent modulator of activated T and B lymphocytes and has been shown in animal models to prevent the onset of diabetes mel-

litis, multiple sclerosis, rheumatoid arthritis, and Crohn's disease.³ Because of the risk of hypercalcemia upon treatment with 1,25(OH)₂D, non-hypercalcemic VDR agonists are being used in patients for treating psoriasis and are being developed for clinical applications for other autoimmune diseases.⁴

Vitamin D Receptor: Basic Science

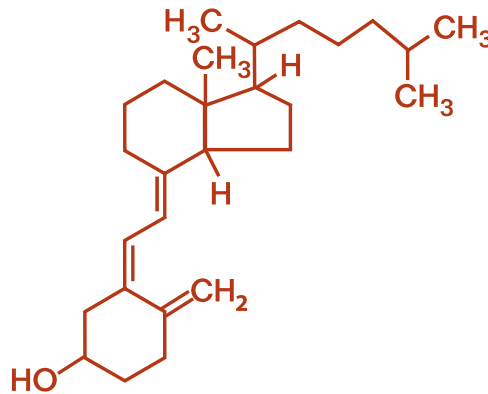
Many vitamin D effects on target tissues are mediated by the VDR, a member of the superfamily of nuclear hormone receptors. Ligand binding induces conformational changes in the receptor, its heterodimerization with the retinoid receptor, and interactions with coactivator proteins, resulting in histone modification at specific gene promoter sites (vitamin D response elements) and transcription of target genes. Many of 1,25(OH)₂D's anti-proliferative, pro-differentiating, and immunomodulatory activities have been shown to involve the VDR.

All osteoblasts have the VDR. The rat osteoblast was a paradigm for detailed research on the structure and function of the vitamin D response element and 1,25(OH)₂D's control of the transcription of the bone-matrix protein osteocalcin, but subsequent work showed that genotropic effects on osteoblasts depend on species and cell maturation.

Vitamin D's systemic effects on calcium and phosphate metabolism had made it difficult to determine whether vitamin D has direct effects on bone formation and mineralization. VDR knockout mice give researchers an opportunity to dissect specific effects on bone.⁵ Most mutant mice died young with hypocalcemia. Maintenance on a rescue diet rich in calcium and phosphorus completely corrected bone formation and mineralization, but alopecia remained. This surprising result indicates that vitamin D may not be needed for bone matrix formation and mineralization in the setting of normal calcium homeostasis. Studies with calvarial osteoblasts from the VDR-knockout mice showed that they had elevated osteoblast and osteogenic activities, compared with wild-type cells. These findings indicate that in mice, VDR absence in fact increases osteoblast differentiation and activity, although how the VDR attenuates osteoblast differentiation is not yet known.⁵

It may seem paradoxical that, in addition to being needed for mineralization of bone matrix, vitamin D is involved in the mobilization of calcium from bone stores. Very low concentrations of 1,25(OH)₂D (~0.1 μg/kg) are needed to

“**Studies show increased prevalence of vitamin D insufficiency in patients with fragility fractures**”



stimulate intestinal absorption of calcium, whereas calcium release from bone requires 10–50 times more 1,25(OH)₂D.⁶ Local factors and PTH levels are likely to influence which process predominates in vivo. 1,25(OH)₂D is a differentiating agent that promotes development of bone-resorbing osteoclasts, and evidence indicates that this is mediated by stimulation of osteoblast/stromal cell production of RANK ligand, the key regulator of osteoclast differentiation. It is notable that in vitro, osteoblasts from VDR-knockout mice cannot support osteoclast differentiation from progenitors in the presence of 1,25(OH)₂D, but can do so in the presence of PTH and interleukin 1α

VDR exists in the small intestine, colon, activated T and B lymphocytes, β-islet cells, keratinocytes, and cells of most

body organs. Recent work on calbindin, the vitamin D target protein in mammalian and avian intestine and kidney, where it serves to facilitate calcium diffusion, indicates that it is present in many other cell types, including osteoblasts and pancreatic β-cells. Evidence shows that calbindin-D_{28k} protects those cells and cells transfected with the calbindin gene against apoptosis—in part by buffering calcium, by reducing free radical formation, and by binding caspase 3, a key mediator of apoptosis.⁷ This implies that sufficient vitamin D and calbindin levels may protect against glucocorticoid-induced apoptosis of osteoblasts and secondary osteoporosis.

VITAMIN D'S EFFECTS ON BONE AND OTHER TISSUES

- Vitamin D deficiency is associated with osteomalacia and osteoporosis.
- Calbindin is a key mediator of 1,25-dihydroxyvitamin D's stimulation of calcium transport in intestine and kidneys.
- Vitamin D is necessary for muscle health and strength.
- Vitamin D receptor mediates the hormone's complex effects in many cell types, including osteoblasts and osteoclasts, and polymorphisms in the vitamin D receptor may account for genetic differences in skeletal metabolism.
- 1,25-dihydroxyvitamin D has potent non-genomic effects on target tissues.

Vitamin D Receptor: Clinical Science

Searches for the genetic basis of bone density or osteoporotic fractures have generated many candidate genes, including polymorphisms in VDR, collagen alpha 1 chain (COL1A1), estrogen receptor α (ER α), interleukin-6, and LDL receptor-related protein 5 (LRP5).⁸ It appears likely that several genes may interact and that different ones may predominate in different ethnic groups, subgroups, or by sex. For example, interactions of polymorphisms of VDR and COL1A1 or VDR and ER α genotypes have been associated with certain fracture sites. In addition, data indicate that patients treated with calcium and vitamin D have different fracture rates, depending upon VDR genotype.⁹

Non-genomic Effects of Vitamin D

1,25(OH)₂D elicits a variety of non-genotropic effects through VDR, including rapid activation of protein kinases and the modulation of the electrical state of cells, including osteoblasts, by acting on membrane calcium and chloride ion channels. Little is known about the mechanism of crosstalk between genomic and non-genomic actions. Some light has been shed on this recently from studies of metabolites and analogs of 1,25(OH)₂D and modified VDRs; the ligand-binding domain of the VDR but not the DNA-binding domain was needed for non-genotropic effects such as kinase activation and anti-apoptosis.¹⁰ These findings raise the possibility that new non-calcemic ligands could be designed with very selective effects on specific targets of native 1,25(OH)₂D.

From the Clinical Scientist:

Vitamin D has long been known to promote calcium absorption and decrease circulating parathyroid hormone levels. Vitamin D supplementation also retards bone loss in older men and women.¹

Low serum levels of 25-hydroxyvitamin D [25(OH)D], the best clinical index of vitamin D status, have recently been associated with several indices of lower extremity strength and performance. In women aged 60 years and older who participated in the National Health and Nutrition Examination Survey III (NHANES III), individuals with lower 25(OH)D levels walked more slowly and were slower to rise from a sitting to a standing position than subjects with 25(OH)D levels at the upper end of the reference range.² Vitamin D receptors are present in muscle tissue³ and may play a role in mediating vitamin D's impact on lower extremity performance. Muscle weakness is a risk factor for falls in the elderly. A recent meta-analysis of randomized, placebo-controlled vitamin D intervention studies suggested that vitamin D lowers the risk of falling by 22% in the elderly.⁴

Given the effects of vitamin D in increasing bone mass and potentially lowering the risk of falling, one would anticipate that supplementation with vitamin D might lower risk of fracture. This question has been examined in several randomized, controlled trials. In two large trials that tested 400 IU of vitamin D₃ per day versus placebo, no fracture risk

reduction was observed.^{5,6} However, in a study that tested 100,000 IU of vitamin D₃ given orally every 4 months (representing a daily dose of 833 IU), the rate of all clinical fractures was significantly lower in the supplemented group than in the placebo group.⁷ Three trials that used combination calcium and vitamin D₃ in daily doses ranging from 500 to 1,200 mg of calcium and 700 to 800 IU of vitamin D₃, supplementation lowered the risk of hip⁸ and all non-vertebral fractures.⁸⁻¹⁰ A recent meta-analysis involving up to 9,820 subjects found that supplementation with 700 to 800 IU per day of vitamin D₃ lowered the risk of hip fracture by 26% and the risk of any non-vertebral fracture by 23% compared with calcium or placebo.¹¹ In addition, these vitamin D doses raised mean serum 25(OH)D levels to 74 nmol/L and higher (range 74–112). No fracture risk reduction was observed among the trials that tested lower doses and in which mean serum 25(OH)D levels reached 54 to 64 nmol/L.¹¹

A recently published trial of an open combination of calcium and vitamin D supplementation (1,000 mg, 800 IU) in 3,314 women aged 70 years and older, for an average of 25 months, showed no significantly lower risk of fracture compared with the control group.¹² The subjects' mean dietary calcium intake was estimated to be 1,100 mg per day and only 60% of the subjects remained on supplements at 12 months. These factors, together with the open design, may have contributed to the negative findings. Another recent study, the RECORD GROUP Trial, had a two-by-two factorial design, and examined the effects of 1,000 mg of calcium, 800 IU of vitamin D₃, the combination, and placebo on fracture rates in 5,292 men and women, aged 70 years and older, over a median period of 45 months.¹³ This was a secondary prevention trial, and all participants had had a fracture in the 10-year period before entry. In this trial, fracture rates in the supplemented groups did not differ significantly from the fracture rate in the placebo group. Only 54% of subjects were taking supplements at 24 months. A small non-random subset of 60 subjects had measurements of serum 25(OH)D and

VITAMIN D: DIETARY SOURCES, NON DIETARY SOURCES, AND THE NEEDED INTAKE

- Low 25-hydroxyvitamin D levels lead to lower bone density and muscle weakness.
- In clinical trials, the mean serum 25-hydroxyvitamin D level achieved during supplementation is linked to impact on fractures.
- Maintaining a 25-hydroxyvitamin D level of 80 nmol/l (32.5 ng/ml) or above is likely to decrease fracture risk.
- An intake of at least 800 to 1000 IU of vitamin D₃ is needed to bring a group mean 25-hydroxyvitamin D level to 80 nmol/l.
- For supplementation, vitamin D₃ is preferred to vitamin D₂.

after 1 year of supplementation with vitamin D₃, the mean 25(OH)D level was approximately 62 nmol/L. This is similar to the mean levels of 54 and 64 nmol/L achieved in the 400 IU supplement trials cited above, which also did not find lower fracture rates on supplementation.^{5,6} Limited compliance and persistent suboptimal 25(OH)D levels during supplementation may have influenced the results in this trial.¹³

In conclusion, further work is needed to fully define the impact of calcium and vitamin D on fracture risk. In the meantime, it appears that maintaining 25(OH)D levels at 75 to 80 nmol/L or higher is likely to reduce the risk of fracture in older men and women.

From the Clinical Practitioner:

Clinicians encounter vitamin D deficiency in a myriad of ways, from the obvious manifestations of hypocalcemia and osteomalacia to subtle changes masquerading as frailty or osteoporosis. The accelerated bone loss in elderly men and women has been attributed to vitamin D deficiency, secondary hyperparathyroidism, and increased osteoclastic bone resorption. Muscle weakness and increased fall frequency are also noted in older adults with vitamin D deficiency.¹ Vitamin D supplements of at least 700 IU per day, with or without calcium have been documented, in several but not all studies, to decrease the risk of fragility fractures, including hip fracture.²⁻⁴

Because the prevalence of vitamin D deficiency defined by serum 25-hydroxyvitamin D levels is higher than previously assumed, all physicians regularly encounter patients in their practice who have inadequate vitamin D intake.⁵ These patients may present in one of several scenarios, some of which are described here.

The Cause of Inexplicably Low Bone Density

The diagnosis of osteoporosis is now made on the basis of bone density values (T-score lower than -2.5 in postmenopausal women). Because BMD tests measure the mineral content of the skeleton (and not the amount of bone tissue), other bone diseases such as osteomalacia and osteogenesis imperfecta can present as low bone density. Patients with celiac disease or after bariatric surgery may have significant malabsorption of vitamin D and calcium without noticeable GI symptoms. In individuals with unexplained low bone density, assessment of vitamin D status is important.

Bone Loss While on Osteoporosis Therapy

In clinical trials, bone loss rarely occurs in healthy postmenopausal women treated with estrogen or bisphosphonates. Observing a statistically significant decrease in BMD

“**Maintaining 25(OH)D levels at 75 to 80 nmol/L or higher is likely to reduce the risk of fracture**”

while on potent antiresorptive drugs should prompt a search for metabolic and medical causes of bone loss. In my referral practice, vitamin D deficiency is the most common explanation for bone loss while on therapy. Correcting the vitamin D deficiency (instead of changing the treatment) usually results in a substantial restoration of bone density.

Pelvic and Sacral Insufficiency Fractures and Poor Fracture Healing

Vitamin D deficiency is known to be associated with insufficiency fractures. Very elderly adults who present with insufficiency fractures of the pelvis, sacrum, and proximal femur deserve evaluation for vitamin D deficiency. Delayed healing of these and other fractures can be a consequence of and a sign of vitamin D deficiency.

Bone Pain and Hypercalcemia upon Initiation of Bisphosphonate Therapy

In the presence of vitamin D deficiency, serum calcium levels are maintained at the expense of secondary hyperparathyroidism and increased bone resorption. Administration of a potent inhibitor of osteoclast activity, such as a bisphosphonate, results in modest, transient decrease in serum calcium in subjects who are vitamin D-replete in clinical trials. In vitamin D-deficient subjects, symptomatic hypocalcemia has been observed.⁶ Bone pain, usually mild and transient, occurs infrequently with bisphosphonate dosing. In some patients who have such effects, I have observed vitamin D deficiency.

Because the prevalence of vitamin D deficiency is so high (more than 50% of healthy women aged 65 years and older in my city), we recommend that all older adults receive supplements of 800–1,200 IU of vitamin D per day.

CLINICAL MANIFESTATIONS OF VITAMIN D DEFICIENCY

- Serum calcium and alkaline phosphatase levels are often normal in patients with Vitamin D deficiency.
- Vitamin D deficiency is a common cause of bone loss in older adults.
- Vitamin D deficiency is an important cause of unexpectedly low bone density in patients of any age.
- Vitamin D deficiency may blunt bone density response to treatment.
- Vitamin D deficiency should be considered in patients with insufficiency fractures or poor fracture healing.

Alternatively, we administer the prescription dose of vitamin D (50,000 units) once each month. In the absence of hypercalcemia or granulomatous disease, there are no contraindications to these doses. Both primary care physicians and specialists have the opportunity to recognize and to correct vitamin D deficiency in older adults. This may be the single most important strategy to reduce the likelihood of non-vertebral fractures. ■

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About the Tri-Point Series

This is the sixth appearance of the tri-point perspective articles in *Endocrine News*. Past series topics have been:

- Obesity
- Polycystic Ovary Syndrome
- Diabetes
- Androgen Therapy for Women
- Cardiovascular Disease

The topics, authors, and outside reviewers are selected by The Endocrine Society's Research Affairs Committee (RAC) to explore subject areas from three different angles—that of the basic researcher, the clinical researcher, and the physician-in-practice. The authors write their articles independently. The drafts are then reviewed by contributing co-editors and by independent experts in the specific topic area.

Endocrine News staff would like to thank the efforts of Dr. Steven Grinspoon, RAC Co-Chair, and Dr. Ellen Seely, Co-Editor, for their dedication in developing this series for our readers.

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* Archived issues of the Tri-Point series can be found on the *Endocrine News* Web site, www.endo-society.org/news/endocrine_news.

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