Symposium: Nutritional Advances in Human Bone Metabolism

Vitamin D and Bone Health[1,2]

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ABSTRACT Vitamin D plays an essential role in maintaining a healthy mineralized skeleton for most land vertebrates including humans. Sunlight causes the photoproduction of vitamin D_3 in the skin. Once formed, vitamin D_3 is metabolized sequentially in the liver and kidney to 1,25-dihydroxyvitamin D. The major biological function of 1,25-dihydroxyvitamin D is to keep the serum calcium and phosphorus concentrations within the normal range to maintain essential cellular functions and to promote mineralization of the skeleton. Most foods do not contain any vitamin D. Foods fortified with vitamin D have a variable amount present and cannot be depended on as a sole source of vitamin D nutrition. Exposure to sunlight provides most humans with their vitamin D requirement. Aging, sunscreen use and the change in the zenith angle of the sun can dramatically affect the cutaneous production of vitamin D_3. Vitamin D insufficiency and vitamin D deficiency is now being recognized as a major cause of metabolic bone disease in the elderly. Vitamin D deficiency not only causes osteomalacia but can exacerbate osteoporosis. It is generally accepted that an increase in calcium intake to 1000–1500 mg/d along with an adequate source of vitamin D of at least 400 IU/d is important for maintaining good bone health. J. Nutr. 126: 1159S–1164S, 1996.

INDEXING KEY WORDS:

- *vitamin D* • *1,25-dihydroxyvitamin D*
- *osteoporosis* • *calcium*

Approximately 400 million years ago as vertebrates ventured from the oceans onto land, they were confronted with a very significant crisis. In their ocean environment, which contained a high calcium content, they utilized this divalent cation for a variety of cellular and metabolic processes. In addition, this divalent cation was a major component of the skeleton that provided its ridged structure. However, on land the environment was deficient in calcium and, as a result, these early vertebrate life forms needed to develop a mechanism(s) to utilize and process the scarce amounts of calcium in their environment to maintain essential cellular and metabolic activities. In addition, they required large amounts of calcium to mineralize their skeleton. For most ocean dwelling animals, the calcium could be easily extracted by specific calcium transport mechanisms in the gills. Once on land, a new strategy developed whereby the intestine evolved to efficiently absorb what little calcium was present in the diet. For reasons that are unknown, an intimate relationship between sunlight and vitamin D evolved to play a critical role in regulating the efficiency of dietary calcium absorption and to maintain a mineralized skeleton.

Photosynthesis of vitamin D_3 in the skin

There is evidence the earliest phytoplankton species that existed in the Sargasso Sea for over 750 million years produced a 5,7-diene sterol (ergosterol; provitamin D_2) that, when exposed to sunlight, was converted to vitamin D_2 (ergocalciferol) (Holick 1989). Whether this photosynthetic process played any significant role in calcium metabolism in these early life forms is unknown. However, some later time in evolution, exposure of the skin of land vertebrates to sunlight resulted in the photosynthesis of the calcium regulating secosteroid vitamin D.

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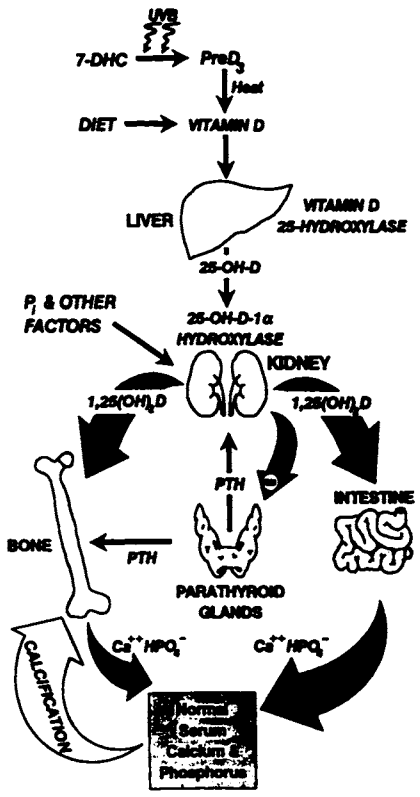


FIGURE 1 During exposure to solar UVB photons, 7-dehydrocholesterol (7-DHC) is converted in the skin to previtamin D_3 (preD_3). Once formed, preD_3 undergoes a thermally induced isomerization to vitamin D_3. Vitamin D (vitamin D_2 or vitamin D_3) from the skin or diet is metabolized sequentially to 25-hydroxyvitamin D (25-OH-D) and 1,25-dihydroxyvitamin D_3 1,25$(OH)_2$D) which, in turn, stimulates intestinal calcium absorption and bone calcium mobilization. Parathyroid hormone (PTH) is one of the major regulators of 1,25$(OH)_2$D production and stimulates calcium mobilization from the bone. The net effect of 1,25$(OH)_2$D is to maintain a normal serum calcium and phosphorus to promote bone mineralization.

7-Dehydrocholesterol (provitamin D_3, 7-DHC)[4] is the immediate precursor for cholesterol biosynthesis in most tissues in the body. In the skin, 7-DHC serves an additional function. 7-Dehydrocholesterol is photolabile and when exposed to high energy ultraviolet B (290–315 nm) radiation from sunlight, this cholesterol precursor is converted to previtamin D_3 (precholecalciferol) (**Fig. 1**) (Holick 1994). Once formed, previtamin D_3 is efficiently converted to vitamin D_3 (cholecalciferol) by a membrane enhanced process (Tian et al. 1993). As vitamin D_3 is being formed, its conformation is altered, resulting in it selectively exiting the plasma membrane of the skin cell into the extracellular space where it eventually enters the circulation and it is bound to the vitamin D-binding protein.

[4] Abbreviations used: 1,25$(OH)_2$]D, 1,25 dihydroxyvitamin D; 7-DHC, 7-dehydrocholesterol; PTH, parathyroid hormone; VDR, vitamin D receptors.

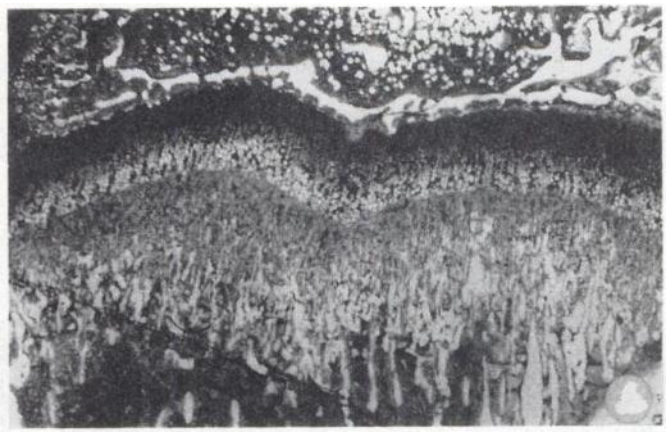
Metabolism and biological activity of vitamin D for calcium metabolism

Once vitamin D_3 is made in the skin or vitamin D_2 and vitamin D_3 are ingested from the diet, the vitamin D (vitamin D without a subscript represents either vitamin D_2 or D_3) is transported to the liver where it is metabolized to its major circulating form, 25-hydroxyvitamin D (25-hydroxycholecalciferol and 25-hydroxyergocalciferol; 25-OH-D) (Darwish and DeLuca 1993, Holick 1995). 25-OH-D is biologically inert on calcium metabolism at physiological concentrations and requires a further hydroxylation in the kidney to form its biologically active metabolite, 1,25-dihydroxyvitamin D [1,25-dihydroxycholecalciferol and 1,25-dihydroxyergocalciferol; 1,25$(OH)_2$D] (Fig. 1).

The major biological function of vitamin D is to maintain the serum calcium in the normal physiological range to preserve neuromuscular and cellular functions. 1,25$(OH)_2$D maintains the blood calcium in the normal range by enhancing the efficiency of intestinal calcium absorption and by increasing the mobilization of stem cells to become osteoclasts that, in turn, mobilize calcium stores from bone (Darwish and DeLuca 1993, Holick 1994, Holick 1995) (Fig. 1). A decrease in the blood-ionized calcium concentration stimulates the parathyroid glands to increase the synthesis and secretion of parathyroid hormone (PTH), which, in turn, increases tubular reabsorption of calcium in the kidney and enhances the production of 1,25$(OH)_2$D (Fig. 1). PTH and 1,25$(OH)_2$D act in concert to mobilize monocytic stem cells to become osteoclasts, thereby increasing calcium removal from the bone(s). 1,25$(OH)_2$D independently increases the efficiency of intestinal calcium absorption. The net effect is to raise serum-ionized calcium concentrations that negatively feedback regulates synthesis and secretion of PTH from the parathyroid glands (Fig. 1). 1,25$(OH)_2$D also independently interacts with the vitamin D receptor in the parathyroid glands and results in an inhibition of the transcription of the PTH gene (Holick 1994). Thus, 1,25$(OH)_2$D plays a critical role in maintaining the blood calcium in the normal range.

Biological function of vitamin D in bone

It is well known that vitamin D deficiency is associated with rickets in children and osteomalacia in adults (Demay 1995, Goldring et al. 1995, Krane and Holick 1994). Before the epiphyseal plates close, vitamin D deficiency causes a disorganization and hypertrophy of the chondrocytes at the mineralization front as well as a mineralization defect (**Fig. 2**), resulting in the short stature and bony deformities that are characteristic of vitamin D deficiency rickets. Osteomalacia, on the other hand, occurs after the epiphyseal plates close; therefore, this disease of adults is more subtle. There



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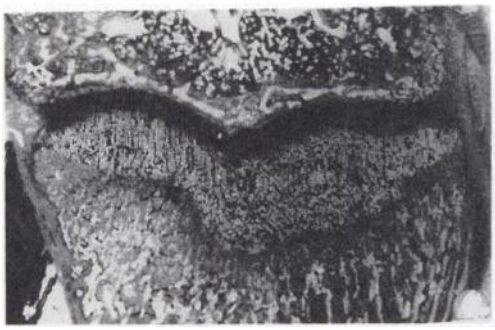

B

C

FIGURE 2 Epiphyseal plates of tibias from rats that were fed (***A***) a vitamin D-deficient diet and supplemented with 125 ng (5 IU) of vitamin D_3 orally five times per wk, (***B***) a vitamin D-deficient diet containing 3% calcium and 0.65% phosphorus and (***C***) a vitamin D-deficient diet with 20% lactose, 4% calcium and 1% phosphorus. Note the wide and disorganized hypertrophic zone in the vitamin D-deficient rat's tibial epiphyseal (***B***) fed high calcium and normal phosphorus diet compared with the normal tibal epiphyseal plates from the rats that were either vitamin D repleted (***A***) or maintained a normal serum calcium and phosphorus by being on a high calcium lactose, high phosphorus diet (***C***). Reproduced with permission from Holtrop et al. (1986).

is a mineralization defect in the skeleton resulting in poor mineralization of the collagen matrix (osteoid) (Goldring et al. 1995, Krane and Holick 1994). Although this does not cause bony deformities, it can cause severe osteopenia (a decrease in the opacity of the skeleton as seen by x-ray) that results in increased risk of skeletal fractures (Aaron et al. 1974, Chalmers et al. 1967, Kavookjian et al. 1990, Sokoloff 1978). In addition, some patients with osteomalacia complain of localized or generalized unrelenting deep bone pain.

It is still not clear exactly what role $1,25(OH)_2D$ has on the bone mineralization process. Osteoblasts, which are responsible for laying down the collagen and protein matrix in the skeleton, possess receptors for vitamin D (VDR). $1,25(OH)_2D_3$ stimulates the synthesis of noncollagenous proteins such as osteocalcin, osteopontin and osteonectin, increases alkaline phosphatase activity and decreases collagen synthesis (Demay et al. 1989, Chang et al. 1994).

There are several studies to support the hypothesis that the principal function of vitamin D in mineralizing bone is through its action on maintaining an adequate calcium × phosphorus product in the circulation and extracellular fluid space. When vitamin D-deficient rats were infused with high calcium and high phosphorus for several days, it was found that the bones had little evidence of rickets (Holick 1995). Similarly, when vitamin D-deficient rats were fed a high calcium, high phosphorus diet, histologic studies of the skeleton did not reveal any skeletal abnormalities consistent with either osteomalacia or rickets (Holtrop et al. 1986) (**Fig. 2**). When a child with severe rickets caused by the rare hereditary disease, vitamin D-dependent rickets Type II [also known as hereditary resistance to $1,25(OH)_2D$, which caused by a genetic defect in the VDR], was infused with calcium for 7 mo, her skeleton began to mineralize normally (Balsan et al. 1986). Therefore, all evidence suggests that the principal function of vitamin

D for maintaining a healthy mineralized skeleton is to ensure that the blood and extracellular concentrations of calcium and phosphorus are adequate for the deposition of calcium hydroxyappatite in the bone matrix that had been laid down by the osteoblasts. There is little evidence to suggest that vitamin D plays a direct role in the bone mineralization process.

Importance of vitamin D for bone health

Vitamin D plays a critically important role in the development, growth and mineralization of the skeleton during its formative years. Vitamin D performs an equally essential role in maintaining a healthy mineralized skeleton for adults of all ages. Vitamin D deficiency in children results in the bone-deforming disease rickets (Demay 1995, Goldring et al. 1995, Holick 1995, Krane and Holick 1994). In adults, vitamin D insufficiency and vitamin D deficiency has a more subtle effect on the skeleton. As the body becomes vitamin D insufficient, the efficiency of intestinal calcium absorption decreases from ~30–50% to no more than 15%. This results in a decrease in the ionized calcium concentration in the blood, which signals the calcium sensor in the parathyroid glands resulting in an increase in the synthesis and secretion of PTH. PTH not only tries to conserve calcium by increasing renal tubular reabsorption of calcium but also plays an active role in mobilizing stem cells to become active calcium resorbing osteoclasts (Fig. 1). PTH also increases tubular excretion of phosphorus causing hypophosphatemia. The net effect of vitamin D insufficiency and vitamin D deficiency is a normal serum calcium, elevated PTH and alkaline phosphatase and a low or low normal phosphorus. The hallmark for vitamin D insufficiency and vitamin D deficiency is low normal (between 10 and 20 ng/ml) and low or undetectable (<10 ng/ml) 25-OH-D, respectively, in the blood. The secondary hyperparathyroidism and low calcium × phosphorus product is thought to be responsible for the increase in unmineralized osteoid, which is the hallmark for rickets and osteomalacia. In addition, the increase in serum PTH causing increased osteoclastic activity results in calcium wasting from the bone, which exacerbates osteoporosis in older adults.

There are several studies that demonstrated an increase in calcium intake of 800–1000 mg/d with supplementation of ≥400–800 units of vitamin D daily will decrease the risk of vertebral and nonvertebral fractures and increase bone mineral density (Chapuy et al. 1992, Dawson-Hughes et al. 1990, Dawson-Hughes et al. 1991, Lips et al. 1988). It has been recognized for over two decades that vitamin D deficiency is associated with increased risk of hip fracture (Aaron et al. 1974, Chalmers et al. 1967, Kavookjian et al. 1990, Sokoloff 1978). It has been reported in several European studies, as well as a study in Boston, that up

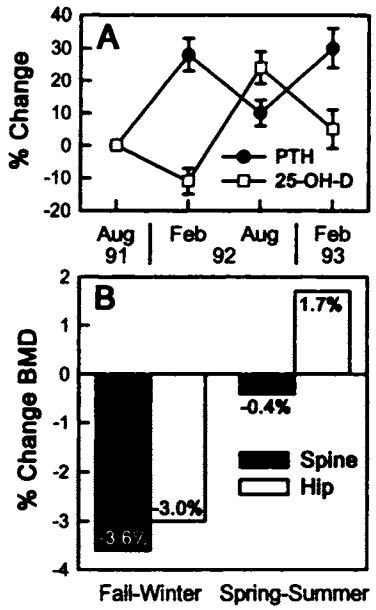

FIGURE 3 (***A***) Seasonal changes (as percentage of previous measurement) in serum parathyroid hormone (PTH) and 25-hydroxyvitamin D (25-OH-D) over 18 mo of the study period. Results are ± SEM produced with permission from Rosen et al. (1994). (***B***) percent change in the bone mineral density of the average L_2-L_4 spine and femoral neck of 15 older rural Maine women during fall and winter and spring and summer.

to 40% of patients who were admitted for an acute hip fracture were vitamin D deficient (Aaron et al. 1974, Kavookjian et al. 1990). During winter when the sunlight loses its ability to produce vitamin D_3 in the skin, there is a more marked loss of bone mineral density of the hip and spine that is related to a decrease in circulating levels of 25-OH-D and an increase in PTH concentrations (Dawson-Hughes et al. 1991, Rosen et al. 1994) (**Fig. 3**).

Sources of vitamin D

Vitamin D is very rare in unfortified foods. Vitamin D in varying amounts is present in the flesh of fatty fish and oils of fish including cod and tuna liver oil (Holick 1989, Holick 1994). Several foods are fortified with vitamin D including milk, some cereals and some bread products. There is preliminary evidence to suggest that meats from poultry, pork and beef contain small amounts of vitamin D that probably comes from the vitamin D that was fortified in the animal feed (Thompson and Plouffe 1993). Although milk is considered to be the major food source of vitamin D, three separate studies have shown that <20% of milk samples evaluated from all sections of the United States and in western Canada contained the amount of vitamin D stated on the label (Chen et al. 1993, Holick et al. 1992, Tanner et al. 1988). In addition, 14% of skim milk samples contained no detectable vitamin D (Holick et al. 1992). Multivitamin preparations that we evaluated contained at

least the amount of vitamin D stated on the label and often contained up to 150% as much, which is important for a good shelf life (Holick 1994).

The major source of vitamin D for most humans is casual exposure to sunlight. It is estimated that upwards of 80–90% of the body's requirement for vitamin D comes from this source (Holick 1994). There are several factors that can affect the cutaneous synthesis of vitamin D_3. Anything that limits the amount of solar ultraviolet B (UVB) photons to reach the skin's surface and penetrate into the viable epidermis can significantly affect this vital photosynthetic process. Melanin, which is a natural sunscreen, clothing and topically applied sunscreens absorb UVB photons and, therefore, can significantly diminish the synthesis of vitamin D_3 (Holick 1994, Matsuoka et al. 1987). The topical application of a sunscreen with a sun protection factor of 8 can almost completely eliminate the cutaneous production of vitamin D_3 (Matsuoka et al. 1987) and cause vitamin D deficiency (Matsuoka et al. 1988). Season, latitude and time of day can significantly affect the cutaneous production of vitamin D_3. When the zenith angle of the sun is so oblique (such as during the winter and at far southern and northern latitudes), the UVB photons are efficiently absorbed in the earth's ozone layer, resulting in little or no production of vitamin D_3 in the skin. In Boston between the months of November and February, exposure to sunlight for up to 5 h does not result in any significant production of vitamin D_3 in the skin (Webb et al. 1989). Aging causes a marked reduction in the cutaneous stores of 7-dehydrocholesterol, resulting in a marked reduction in the production of vitamin D_3. By the age of 70 y, the skin's ability to produce vitamin D_3 is only 30% as efficient as when the individual was a young adult (Holick et al. 1989).

The skin has a large capacity to produce vitamin D_3. For a young adult a whole body exposure to one minimal erythemal dose of sunlight can raise the blood levels of vitamin D_3 to a level comparable with taking an oral dose of vitamin D_2 of between 10,000 and 25,000 IU (Holick 1994). When the elderly were asked to sit on a verandah during the spring, summer and fall in New Zealand for 15 or 30 min a day, Reid et al. (1985) demonstrated that there was a substantial increase in circulating concentrations of 25-OH-D (**Fig. 4A**). Furthermore, despite the age-related decrease in the cutaneous production of vitamin D_3, people over the age of 60 y still benefit from limited exposure to sunlight. There was a modest seasonal increase in 25-OH-D in the summer even in adults over 60 y of age (Lund and Sorensen 1979, McKenna et al. 1985, Webb et al. 1990) (Fig. 4B).

It is reasonable for adults over the age of 50 to obtain their vitamin D requirement by being exposed to suberythemal doses of sunlight. I have recommended for adults in Boston that exposure of hands, face and arms two to three times a week to suberythemal doses of sunlight (~5–15 min/d, depending on the skin's sensitivity to sunlight) is adequate to provide sufficient

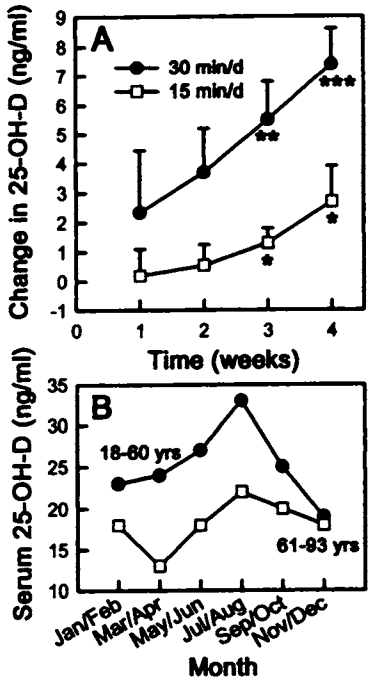

FIGURE 4 (*A*) Change in serum 25-OH-D levels from baseline in elderly rest home residents in Auckland, New Zealand (37°C) spending 15 or 30 min/d outdoors in the spring to expose their heads, necks, forearms and lower legs to sunlight. $n = 5$ each group; $^*P < 0.06$, $^{**}P < 0.02$ and $^{***}P < 0.005$. Adapted from Reid et al. (1985). (*B*) Seasonal variation in serum 25-OH-D levels in Denmark. Adapted from Lund and Sorenson (1979).

amounts of vitamin D_3. Because excess vitamin D_3 that is produced in the skin is stored in the body fat, vitamin D is available during the winter when the sun is incapable of producing vitamin D_3 in the skin. Because a topical application of a sunscreen can essentially prevent the production of vitamin D_3 in the skin, people who wish to stay outdoors for long periods of time should only expose their skin to suberythemal amounts of sunlight and then topically apply a sunscreen with a sun protection factor of 15 or greater to prevent the consequences of chronic excessive exposure to sunlight. For children and young adults, they should wear a sunscreen at all times to help prevent skin damage and skin cancer. Because children and young adults will not always wear a sunscreen over all sun exposed areas, they are still able to produce enough vitamin D_3 from sun exposure to satisfy their body's requirement. A multivitamin that contains 400 IU of vitamin D is an excellent source of the vitamin and will help maintain circulating concentrations of 25-OH-D. However, in the absence of sunlight, a multivitamin may not be adequate to maintain a normal vitamin D status (Holick 1994). There is mounting evidence that in the absence of sunlight the body may need 600–800 IU of vitamin D (Chapuy et al. 1992, Dawson-Hughes et al. 1991, Holick 1994). Although milk, some cereals and bread may contain some vitamin D, they cannot be

depended on as a sole source of vitamin D. People should not take more than one multivitamin pill that contain vitamin D because of concern for vitamin A intoxication (a multivitamin pill usually contains 10,000 units of vitamin A). For patients who are vitamin D insufficient or vitamin D deficient, I usually treat them once a week with 50,000 IU of vitamin D_2 for 8 wk. The serum 25-OH-D usually increases from <15 ng/ml to 25–40 ng/ml. This treatment will maintain a normal vitamin D status for 2–4 mo. Therefore, an adequate source of calcium in combination with vitamin D from sunlight and/or a multivitamin containing vitamin D and exercise ultimately results in good bone health.

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