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Review

Vitamin D: Its role in cancer prevention and treatment

Michael F. Holick*

Boston University Medical Center, 715 Albany Street, M-1013, Boston, MA 02118, USA

Abstract

Vitamin D, the sunshine vitamin, has been recognized for almost 100 years as being essential for bone health. Vitamin D provides an adequate amount of calcium and phosphorus for the normal development and mineralization of a healthy skeleton. Vitamin D made in the skin or ingested in the diet, however, is biologically inactive and requires obligate hydroxylations first in the liver to 25-hydroxyvitamin D, and then in the kidney to 1,25-dihydroxyvitamin D. 25-Hydroxyvitamin D is the major circulating form of vitamin D that is the best indicator of vitamin D status. 1,25-dihydroxyvitamin D is the biologically active form of vitamin D. This lipid-soluble hormone interacts with its specific nuclear receptor in the intestine and bone to regulate calcium metabolism. It is now recognized that the vitamin D receptor is also present in most tissues and cells in the body. 1,25-dihydroxyvitamin D, by interacting with its receptor in non-calcemic tissues, is able to elicit a wide variety of biologic responses. 1,25-dihydroxyvitamin D regulates cellular growth and influences the modulation of the immune system. There is compelling epidemiologic observations that suggest that living at higher latitudes is associated with increased risk of many common deadly cancers. Both prospective and retrospective studies help support the concept that it is vitamin D deficiency that is the driving force for increased risk of common cancers in people living at higher latitudes. Most tissues and cells not only have a vitamin D receptor, but also have the ability to make 1,25-dihydroxyvitamin D. It has been suggested that increasing vitamin D intake or sun exposure increases circulating concentrations of 25-hydroxyvitamin D, which in turn, is metabolized to 1,25-dihydroxyvitamin D₃ in prostate, colon, breast, etc. The local cellular production of 1,25-dihydroxyvitamin D acts in an autocrine fashion to regulate cell growth and decrease the risk of the cells becoming malignant. Therefore, measurement of 25-hydroxyvitamin D is important not only to monitor vitamin D status for bone health, but also for cancer prevention.

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*Corresponding author. Tel.: +1 617 638 4545; fax: +1 617 638 8882.

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5 1. Sources and metabolism of vitamin D

7 Very few foods naturally contain vitamin D (Vieth, 2005; Holick, 2004a, b; Holick, 2002a, b). This is the
 9 reason why at the turn of the 20th century, more than 80% of children living in the industrialized cities of
 Northern Europe and Northeastern United States were crippled by the bone deforming disease, rickets.
 Typically, oily fish including salmon, mackerel and herring; cod-liver oil and sun-dried mushrooms, contain
 11 approximately 300–500 IU of either vitamin D₃ or vitamin D₂ per serving (Vieth, 2005; Holick, 2004a, b;
 Holick, 2002a, b) (D represents D₂ and or D₃). The major foods in the United States that are fortified with
 13 vitamin D include milk, orange juice, and some cereals, bread, yogurt and cheeses. In Europe the fortification
 of various foods including dairy products, custards was routinely practiced until the late 1940s when an
 15 outbreak of vitamin D intoxication resulted in laws being passed to forbid the fortification of milk and a wide
 variety of other products with vitamin D. Most European countries permit margarine and some cereals to be
 17 fortified with vitamin D, and a few countries including Sweden also permit milk to be fortified with this
 vitamin.

19 The action of sunlight on the skin resulting in the production of vitamin D₃ is responsible for most
 (90–95%) of peoples' vitamin D requirement (Holick, 2004a, b; Holick, 2002a, b). During exposure to sunlight
 21 it is the ultraviolet B portion (with energies between 290 and 315 nm) that is absorbed by 7-dehydrocholesterol
 in the epidermis and dermis that results in its conversion to previtamin D₃ (Fig. 1). Previtamin D₃ is rapidly
 23 isomerized by the body temperature to vitamin D₃. Once formed, vitamin D₃ is ejected out of the plasma
 membrane into the extracellular space where it enters the dermal capillary bed bound to the vitamin-D-
 25 binding protein.

Vitamin D₃ from the skin and vitamin D₂ and vitamin D₃ from the diet are metabolized in the liver to 25-
 27 hydroxyvitamin D [25(OH)D]. 25(OH)D is the major circulating form of vitamin D that is used by clinicians
 to determine the vitamin D status of their patients. However, 25(OH)D is biologically inert and requires an
 29 additional hydroxylation in the kidney to form the biologically active form of vitamin D; 1,25-
 dihydroxyvitamin D [1,25(OH)₂D] (Fig. 1) (DeLuca, 2004; Holick, 2004a, b; Holick, 2002a, b).

31 1,25(OH)₂D is a lipid-soluble hormone that interacts with its vitamin D receptor (VDR) in the small
 intestine to increase the expression of an epithelial calcium channel, calcium-binding protein and a variety of
 33 other proteins to help the transport of calcium from the intestinal lumen into the circulation. 1,25(OH)₂D also
 interacts with its VDR in the osteoblasts, which stimulates the expression of the receptor activator of NFκB
 35 ligand (RANKL). This results in a cascading effect to increase the mobilization of osteoclast precursors to
 become mature osteoclasts, which in turn, mobilize calcium stores from the skeleton to maintain calcium
 37 homeostasis (DeLuca, 2004; Holick, 2004a, b; Holick, 2002a, b; Khosla, 2001; Norman et al., 2002).

39

41 2. Role of 1,25(OH)₂D in regulating cell growth

In 1979, Stumpf et al. (1979) reported that not only the intestine, bone and kidney had a VDR, but also that
 43 it appeared that essentially all tissues in the body recognized 1,25(OH)₂D. Shortly thereafter, a variety of
 studies revealed that the skin, colon, prostate, breast, heart, skeletal muscle, brain, monocytes and activated T
 45 and B lymphocytes all expressed VDR. The first insight into the potential non-calcemic role of 1,25(OH)₂D on
 cellular activity was reported by Suda et al. (1982) when they observed that the growth of M-1 leukemic cells
 47 that had a VDR was markedly inhibited by 1,25(OH)₂D₃. In addition, they observed that 1,25(OH)₂D₃
 induced M-1 cell differentiation. This was quickly followed by the observation that human HL-60 leukemic
 49 cells that had a VDR responded in a similar manner (Tanaka et al., 1982). Since these insightful observations,
 it has been observed that cultured breast, colon, prostate, skin, lung and a variety of other cell lines, when
 51 exposed to 1,25(OH)₂D₃, had marked inhibition of cellular growth and induction of terminal differentiation
 (DeLuca, 2004; Feldman et al., 2000; Holick, 2004a, b; Holick, 2002a, b; Norman et al., 2002).

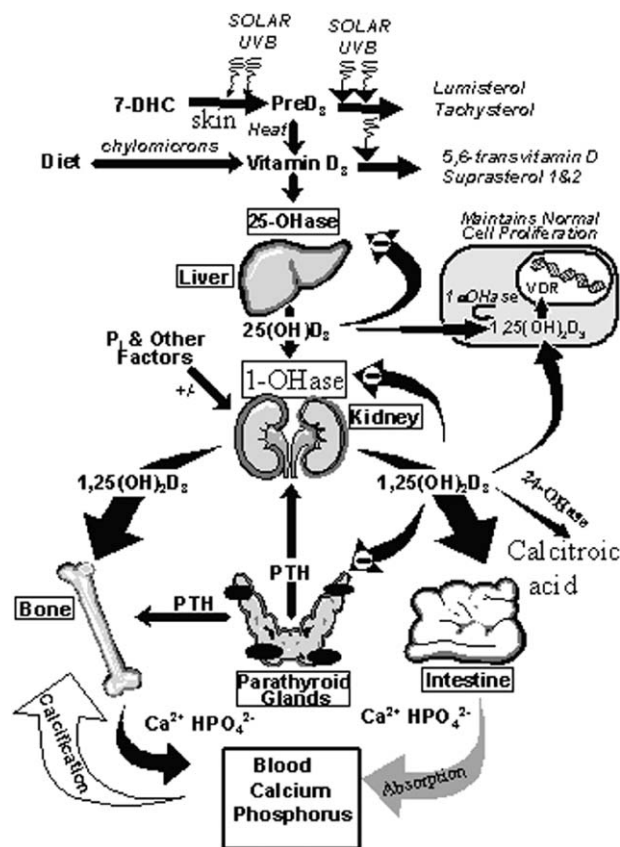


Fig. 1.

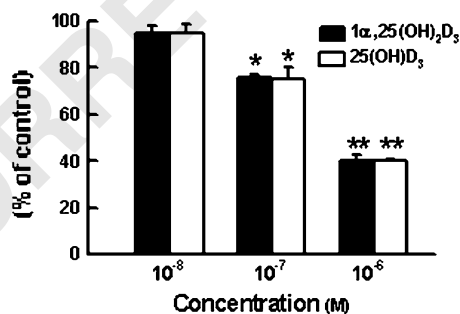


Fig. 2.

In addition, cancer cell lines of the prostate, colon, breast, lung and melanoma that had a VDR had marked inhibition of cellular growth when exposed to 1,25(OH)₂D (Colston et al., 1981a, b; Feldman et al., 2000; Holick, 2004a, b; Holick, 2002a, b; Skowronski et al., 1993) (Fig. 2).

There was great excitement about the possibility of developing 1,25(OH)₂D and its analogs for the treatment of cancer. Koefler et al. (1985) initiated one of the first studies and reported significant remission in patients suffering from preleukemia who were treated with 1,25(OH)₂D₃. Unfortunately, the patients not only developed hypercalcemia, but also became unresponsive to the antiproliferative and prodifferentiating effects of 1,25(OH)₂D₃, and ultimately succumbed to their illness. This put a significant damper on the development

1 of vitamin D analogs for the treatment of cancer. However, it was also appreciated that epidermal cells when,
 2 exposed to $1,25(\text{OH})_2\text{D}_3$ in culture, had marked inhibition of their growth and they terminally differentiated.
 3 Thus, it was reasoned that $1,25(\text{OH})_2\text{D}_3$ and its analogs could be developed as effective treatment for the
 4 hyperproliferative epidermal disorder, psoriasis (MacLaughlin et al., 1985). $1,25(\text{OH})_2\text{D}_3$, calcipotriene, $1,24$ -
 5 dihydroxyvitamin D_3 and $22\text{-oxo-}1,25\text{-dihydroxyvitamin D}_3$ have been developed as effective antipsoriatic
 6 agents. Thus, the antiproliferative effectiveness of $1,25(\text{OH})_2\text{D}_3$ and its analogs for the treatment of
 7 hyperproliferative disorders was established (Holick, 1998).

9 3. The sunlight-vitamin D cancer conundrum

11 In 1941 Apperly (1941) made a curious observation, i.e. people living in northern latitudes in the United
 12 States including Vermont, New Hampshire and Massachusetts were more likely to die of cancer than adults
 13 living in Alabama, South Carolina and Texas as well as other southern states. He did note, however, that
 14 people living in southern states were more likely to develop non-life threatening skin cancer, and suggested
 15 that this provided an immunity for the more serious deadly cancers of the breast, colon and prostate. This
 16 insightful observation went unnoticed until the late 1980s when Garland et al. (1991, 1989) reported that colon
 17 cancer mortality was much higher in Northeastern United States compared to people living in southern states.
 18 It is now well documented that the risk of developing and dying of prostate, breast, colon, ovarian,
 19 esophageal, non-Hodgkin's lymphoma and a variety of other lethal cancers is related to living at higher
 20 latitudes (Bertone-Johnson et al., 2005; Freedman et al., 2002; Garland et al., 1991, 1990, 1989, 1985; Grant,
 21 2004, 2002a, 2002b; Janowsky et al., 1999; John et al., 1999; Lefkowitz and Garland, 1994; Tangrea et al.,
 22 1997; Zhao and Feldman, 2001).

23 The simple explanation for why exposure to sunlight decreased risk of common cancers was that by
 24 increasing the production of vitamin D_3 in the skin resulted in higher circulating levels of $25(\text{OH})\text{D}_3$, which
 25 could be metabolized by the kidney to $1,25(\text{OH})_2\text{D}_3$. Thus, by increasing circulating levels of $1,25(\text{OH})_2\text{D}_3$, it
 26 could interact with the other tissues in the body that had a VDR, which helped maintain cellular growth and
 27 to prevent the cells from becoming malignant. Although the exact mechanism for how $1,25(\text{OH})_2\text{D}$
 28 accomplishes this is not well understood, it has been reported that $1,25(\text{OH})_2\text{D}$ markedly inhibited a variety of
 29 genes responsible for proliferation including p21 and p27, and was also responsible for enhancing apoptosis
 30 genes, and a variety of genes that regulate cellular differentiation (Chen and Holick, 2003; Feldman et al.,
 31 2000; Holick, 2004a, b; Holick, 2002a, b). This all seemed to make sense except for the fact that the kidney's
 32 production of $1,25(\text{OH})_2\text{D}_3$ is exquisitely regulated by parathyroid hormone, serum calcium and phosphorus
 33 and several other hormones (Holick, 2004a, b; Holick, 2002a, b). Thus, increasing vitamin D intake or
 34 increasing sun exposure does not result in increased circulating levels of $1,25(\text{OH})_2\text{D}_3$.

35 In the 1980s cultured keratinocytes and a variety of other cultured cells were reported to have the enzymatic
 36 machinery to produce $1,25(\text{OH})_2\text{D}$ (Bikle et al., 1986). However, the importance of these observations was not
 37 fully appreciated until Schwartz et al. (1998) reported that prostate cells obtain from prostate biopsies
 38 converted $25(\text{OH})\text{D}_3$ to $1,25(\text{OH})_2\text{D}_3$. Since this observation, it has been observed that the colon, lung, breast
 39 among other tissues all express the 25-hydroxyvitamin D-1 α -hydroxylase (1-OHase; cyp 27 B) (Cross et al.,
 40 2001; Mawer et al., 1994; Tangpricha et al., 2001). Thus, it has been suggested that raising blood levels of
 41 $25(\text{OH})\text{D}$ provides an adequate substrate for the prostate, colon and breast to make their own $1,25(\text{OH})_2\text{D}$,
 42 which, in turn, is capable of regulating a variety of cellular processes that helps to keep in check cellular
 43 growth and prevent malignancy. Once $1,25(\text{OH})_2\text{D}$ carries out this important function, it induces its own
 44 destruction by regulating the 25-hydroxyvitamin D-24-hydroxylase (cyp 24) (Omdahl et al., 2002). This
 45 enzyme hydroxylates $1,25(\text{OH})_2\text{D}$ on carbon-24 that initiates a cascade of oxidative events resulting in the
 46 production of the water-soluble and biologically inactive calcitric acid, which is excreted by the kidney into
 47 the urine (Fig. 1).

49 4. Sunlight, vitamin D and cancer prevention

51 The observations by Garland et al. (1991, 1989, 1985), Hanchette and Schwartz (1992), and Grant et al.
 (2002a,b, 2004), set the stage for linking living at higher latitudes and being vitamin D deficient with increased

1 risk of common deadly cancers. It was reported that in both prospective and retrospective studies that if
25(OH)D was at least 50 nmol/L (20 ng/mL) that there was a decreased risk of developing prostate, breast and
3 colon cancer by 30–50% (Garland et al., 1989; Ahonen et al., 2000). It was suggested by Grant that 25% of
breast cancer mortality in Europe was due to living at higher latitude and being vitamin D deficient (Grant,
5 2002a).

The initial observation by Apperly (1941) has been confirmed by several investigators, most recently Grant
7 who reported that increased sun exposure decreases mortality due to common cancers in both white males and
females (Grant, 2002b; Grant and Holick, 2005). These observations are also supported by Luscombe et al.
9 (2001) who reported that men who had little sun exposure developed prostate cancer 3–5 years early than men
who had the most sun exposure.

11 We also evaluated colon cancer incidence in California because the state has wide range of latitudes. We
observed that living in San Diego significantly decreased the risk of developing colon cancer compared to
13 Californians living in San Francisco and further north (Spina et al., 2005).

However, it remained to be determined whether vitamin D deficiency per se increased tumor growth. To
15 evaluate this, Tangpricha et al. (2005) conducted a study whereby they evaluated the growth of a mouse colon
cancer cell line MC-26 in Balb/c mice that were vitamin D deficient or vitamin D sufficient. The cells were
17 implanted in the backs of both groups of animals and by day 9, the tumors became apparent. By day 10, there
was a statistically significant difference in the tumor size in the mice that were vitamin D deficient compared to
19 the group that were vitamin D sufficient. The tumors grew much more rapidly in the vitamin D deficient mice,
and by the end of the study on day 19, the tumors in the mice that were vitamin D deficient were on average
21 80% larger than in the mice that were vitamin D sufficient. The 25(OH)D levels in the vitamin D deficient mice
at the end of the study was < 5 ng/mL, whereas the vitamin D sufficient mice maintained a 25(OH)D of 35 ng/
23 mL. This observation provides strong corroborating data that supports the concept that vitamin D sufficiency
is important for reducing tumor cell growth.

25 Whitlatch et al. (2002) conducted a study that provided strong evidence that the local production of
1,25(OH)₂D₃ in prostate cells is important for regulating prostate cancer cell growth and differentiation. They
27 grew a prostate cancer cell line LNCaP, which has a VDR but has no 1-OHase activity. These cells had
marked inhibition of cellular growth when incubated with 1,25(OH)₂D₃. These cells are unresponsive to
29 25(OH)D₃. The LNCaP cells were transfected with the 1-OHase gene or its antisense counterpart. The cells
transfected with the 1-OHase gene were able to metabolize ³H-25(OH)D₃ to ³H-1,25(OH)₂D₃. When the 1-
31 OHase transfected LNCaP cells were treated with 25(OH)D₃, there was marked inhibition of cell growth. The
normal LNCaP cells and the LNCaP cells transfected with either an empty vector or the antisense gene
33 showed no antiproliferative activity in response to 25(OH)D₃ (Fig. 3). These data strongly support the theory
that by raising blood levels of 25(OH)D₃ that this provides adequate substrate for the 1-OHase that is present
35 in the breast, colon, prostate and other tissues once it is metabolized to 1,25(OH)₂D₃ it modulates cellular
growth and prevents malignancy (Fig. 4).

37 5. Vitamin D fortification and the vitamin D intoxication scare

39 In the late 1920s and early 1930s when it was appreciated that exposure to sunlight or artificial ultraviolet B
41 radiation (UV) imparted antirachitic activity, this led to the ultraviolet irradiation of a wide variety of foods to
impart antirachitic activity and help prevent rickets in children. Once vitamin D was discovered and
43 synthesized on a commercial basis, the UV irradiation of foods was halted and vitamin D fortification was
instituted. A wide variety of foods and drinks were fortified with vitamin D including milk, custard, soft
45 drinks, bread, hot dogs and even beer (Holick, 2002b). After World War II, there was an outbreak of
hypercalcemia in infants in Great Britain that was found to be caused by the over fortification of milk with
47 vitamin D resulting in vitamin D intoxication (British Pediatric Association, 1956). Because vitamin D
analysis in foods was done as a bioassay that at times was unreliable, there was great concern in Great Britain
49 and the rest of Europe about the potential for widespread vitamin D intoxication of infants that could lead to
serious medical problems including mental retardation and kidney failure (Bauer and Freyberg, 1946; British
51 Pediatric Association, 1956). As a result, laws were passed throughout Europe that forbid not only the
fortification of milk and other dairy products with vitamin D, but also almost all foods and even skin creams

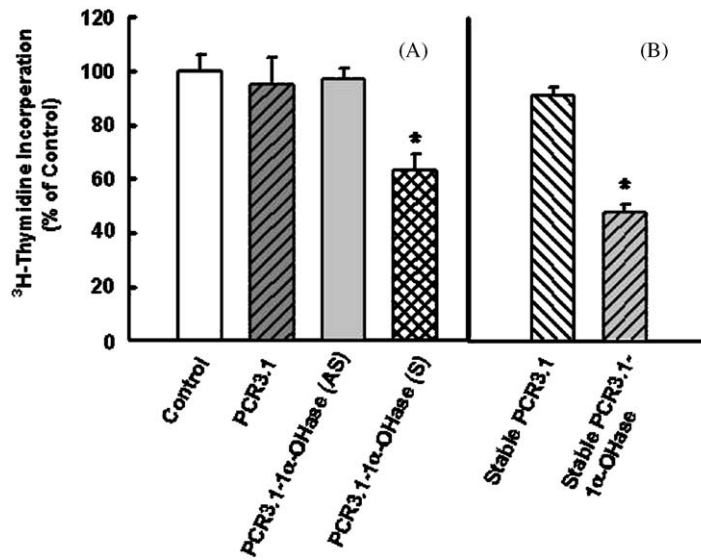


Fig. 3.

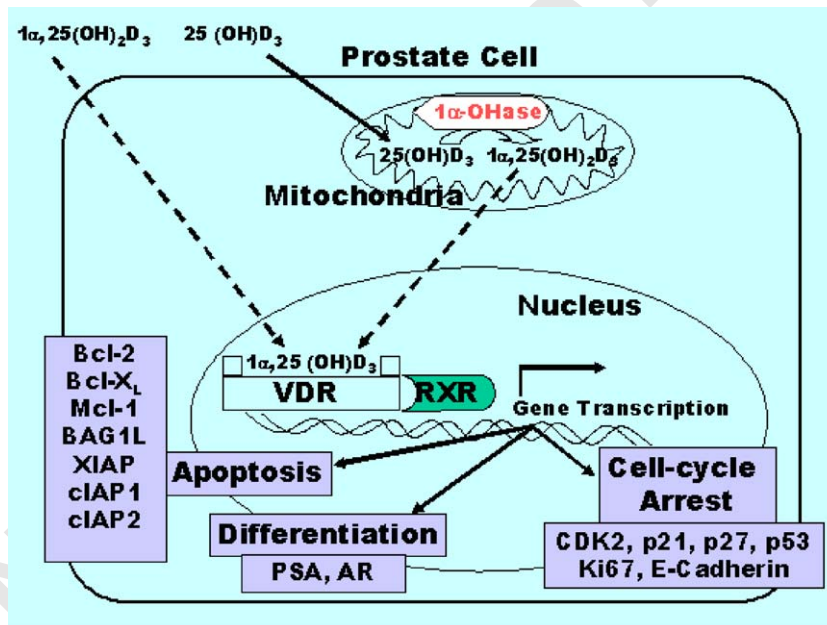


Fig. 4.

with either vitamin D or its precursors. These regulations to forbid the fortification of most foods with vitamin D remain active in most European countries. However, the recognition that vitamin D deficiency is a health risk for children in Europe has resulted in many countries fortifying margarine and some cereals with vitamin D. A few countries have resurrected the fortification of milk with vitamin D including Sweden.

Vitamin D intoxication is caused by excessive ingestion for a prolonged period of time of super-pharmacologic doses of vitamin D. It is worth noting that sun exposure or exposure to ultraviolet radiation

1 will not cause vitamin D intoxication because the ultraviolet radiation destroys any excess vitamin D that is produced (Holick, 2004a, b; Holick, 2002a, b).

3 Vitamin D intoxication is associated with a markedly elevated level of 25(OH)D and is accompanied with hypercalcemia, hyperphosphatemia and hypercalciuria. The Institute of Medicine in 1997 in the United States
5 recommended that the safe upper limit for vitamin D intake for children under 1 year of age was 1000 IU/day and for adults 2000 IU/day. Vieth (1999) reported that healthy male adults who received 1000 or 4000 IU of
7 vitamin D₃ a day for 2–5 months showed no signs of vitamin D intoxication, i.e. the serum and urinary calcium levels remained normal.

9 Vitamin D intoxication is an extremely rare occurrence and is often caused either intentionally or inadvertently because of either poor record keeping or human error. Some examples include the intentional or
11 accidental mixing of sugar with vitamin D₃. The blood levels of 25(OH)D exceeded 3700 nmol/L, and was associated with hypercalcemia (Vieth et al., 2002). Koutkia et al. (2001a) reported a healthy male who was
13 thought to be ingesting 2000 IU of vitamin D₃ from a powdered product. Unfortunately, the manufacturer forgot to dilute it, and therefore, he was ingesting 2 teaspoons of pure crystalline vitamin D₃ a day, which
15 equaled more than 1 million units a day. His 25(OH)D was over 1250 nmol/L (500 ng/mL) and was associated with hypercalcemia. Typically, a 25(OH)D needs to be above 370 nmol/L (150 ng/mL) before the consequences
17 of vitamin D intoxication, i.e. hypercalcemia and decrease in renal function are observed. People most sensitive to a high intake of vitamin D are those with mild to moderate renal failure or those who suffer from
19 chronic granulomatous disorders such as sarcoidosis.

21 6. The development of vitamin D analogs for cancer treatment

23 The major stumbling block in developing vitamin D analogs for the treatment of cancer was the difficulty in separating the calcemic activity of vitamin D analogs from its antiproliferative activity. More than a thousand
25 analogs of vitamin D have been made to date (Bouillon et al., 1995). Several analogs have been effectively used as antiproliferative agents for the treatment of psoriasis. However, to date, there has been scarcity of clinical
27 trials demonstrating the therapeutic efficacy of vitamin D analogs. The one promising study was the observation that the vitamin D analog seocalcitol was effective in treating inoperable hepatocellular
29 carcinoma (Dalhoff et al., 2003).

31 Recently, vitamin D analogs that have an additional side chain known as Gemini analogs have been found to be 100–1000 times more potent in their antiproliferative activity than the natural hormone. Studies in mice
33 suggest that they may be useful in the treatment of some cancers including colon cancer (Spina et al., 2005).

35 7. Recommendations

37 Vitamin D deficiency is now recognized as a worldwide epidemic. Studies in the United States, Europe, India and Southeast Asia all reverberate the same theme, upwards of 50% of both children and adults are at high risk of vitamin D deficiency (Chapuy et al., 1997; Gordon et al., 2004; Kauppinen-Mäkelin et al., 2001; Kinyamu et al., 1998; Lips et al., 2001; Looker et al., 2002; Malabanan et al., 1998; Outila et al., 2001; Sullivan et al., 2005). This is not at all surprising when it is appreciated that humans evolved in sunlight and like many
41 other vertebrates, including non-human primates, have depended on sun exposure for their vitamin D requirement. Most humans on earth no longer are exposed to a sufficient amount of sunlight to satisfy their
43 bodies' requirement for vitamin D. Barger-Lux et al. (1998) has suggested that the body uses between 3000 and 5000 IU of vitamin D₃ a day to satisfy all of its needs. Vitamin D supplementation is not widely practiced
45 and most supplements only contain 400 IU of vitamin D. Most experts now agree that 1000 IU of vitamin D₃ is needed daily in the absence of sun exposure to maintain a healthy blood level of 25(OH)D of between 75 and
47 125 nmol/L (30–50 ng/mL) (Tangpricha et al., 2003).

49 Chuck et al. (2001) reported that a multivitamin supplement for nursing home residence in the UK was less effective than having the residents be exposed to ultraviolet radiation for maintaining adequate blood levels of 25(OH)D. For children and adults who have intestinal fat malabsorption syndromes, often sun exposure and
51 exposure to ultraviolet B radiation from a light source is the only way they can obtain their vitamin D requirement (Koutkia et al., 2001b). Exposure to sensible sunlight and tanning bed radiation is also an

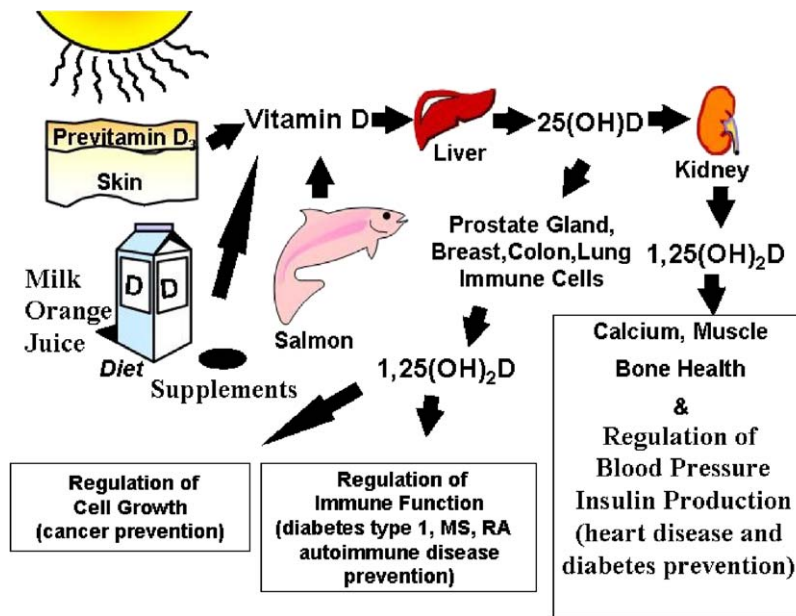


Fig. 5.

effective way of maintaining healthy levels of 25(OH)D (Chel et al., 1998; Reid et al., 1985; Tangpricha et al., 2004).

There needs to be a recognition that sensible sun exposure followed by good sun protection, should be encouraged. Both the United States and Europe should consider increasing fortification of foods with vitamin D. In particular, in Europe there should be a resurrection of fortification of dairy products with vitamin D. In the United States and Europe, fortification of additional foods such as pasta will have important positive health ramifications that are likely to be similar to what was observed when pasta was fortified with folate to decrease the risk of birth defects.

8. Conclusion

The epidemic of vitamin D deficiency has both devastating consequences for bone health and insidious and deadly consequences for overall health and well-being (Heaney, 2003; Holick, 2004a, b; Holick, 2002a, b) (Fig. 5). The link between vitamin D deficiency (inadequate sun exposure) and increased mortality to cancer is now well established. The recent observation by Woo et al. (2005) shows that increasing vitamin D intake decreased prostate-specific antigen levels in men with metastatic prostate cancer should be the impetus to encourage increased intake of vitamin D worldwide. Vitamin D₃ is the preferred form of vitamin D because it is the natural form of vitamin D made in the skin and is approximately 50–80% more effective than vitamin D₂ in maintaining 25(OH)D levels. Children over the age of 1 year and all adults should receive 1000 IU of vitamin D₃ a day, or have sensible sun exposure to satisfy their vitamin D requirement. Measurement of 25(OH)D₃ should be encouraged and should be party of an annual physical exam since there is no other method to determine vitamin D status of either a child or an adult.

Acknowledgements

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