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Review

Dysfunction of the Vitamin D Endocrine System as Common Cause for Multiple Malignant and other Chronic Diseases

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Abstract. *Extensive research on the CYP27B1-encoded 25-(OH)D-1α-hydroxylase has contributed much to our understanding about how locally produced 1,25-$(OH)_2D_3$ exerts tissue-specific control of cellular growth, differentiation and function. Because many types of epithelial, mesenchymal and immune cells express the 25-(OH)D-1α-hydroxylase, many organ functions are necessarily affected by changes in the activity of the enzyme. It is hypothesized that this is likely to occur under conditions of hypovitaminosis D, i.e., at serum 25-(OH)D levels below 30 nM, because extra-renal 25-(OH) D-1α-hydroxylase activity is critically limited by the availability of its substrate. This can provide an explanation, on a molecular and cellular basis, for the many observations that significant associations exist between a compromised vitamin D status and the pathogenesis of frequent chronic diseases. In addition to skeletal disorders, vitamin D insufficiency is a risk factor for malignancies, particularly of the colon, breast and prostate gland, as well as for chronic inflammatory and autoimmune diseases (insulin-dependent diabetes mellitus, inflammatory bowel disease, multiple sclerosis, etc.).*

1,25-Dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), the hormonally active metabolite of vitamin D_3, is a key regulator of cellular proliferation, differentiation and function in many biological systems. 1,25-$(OH)_2D_3$ is synthesized mainly in the kidney from its immediate precursor, 25-hydroxyvitamin D_3 (25-$OH)D_3$, which itself is produced in the liver from vitamin D_3. Importantly, 1,25-$OH)_2D_3$ is also synthesized at a number of extra-renal sites by cell types that express the *CYP27B1*-encoded enzyme, 25-(OH)D-1α-hydroxylase. CYP27B1 activity has been localized, for example, in macrophages and dendritic cells (1, 2), colonocytes (3-5), osteoblasts (6), synovial cells (7), keratinocytes (8), hair follicles, adrenal medulla, pancreatic islets (9), and vascular endothelial cells (10). There is evidence, from both observational studies and clinical trials, that hypovitaminosis D, *i.e.,* a condition of inadequate vitamin D supply, is associated with, notwithstanding skeletal disorders, multiple types of common chronic diseases, such as various cancers, chronic inflammatory and autoimmune diseases, as well as metabolic disorders (for review, (11, 12)). It was the aim of the present review to provide a better understanding of the way in which hypovitaminosis D causes dysfunction of the vitamin D endocrine system, particularly in extra-renal tissues and cells (as exemplified in Figure 1), and thereby increases the risk for chronic diseases of different etiology.

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Vitamin D Status

Hypovitaminosis D denotes a compromised vitamin D status as a result of insufficient cutaneous synthesis or low dietary intake of vitamin D. The serum level of the liver metabolite, 25(OH)D, is considered to be a reliable indicator of the vitamin D status of a given individual. Circulating 25-(OH)D concentrations range between 25-125 nM in the winter months, and between 50-300 nM in summer time. Whereas there seems to be agreement that outright vitamin D deficiency, causing rickets or osteomalacia, is defined by 25-(OH)D concentrations below 10 nM, the cut-off point between vitamin D insufficiency, *i.e.*, sub-optimal vitamin D supply, and vitamin D sufficiency is believed to be in the 25-80 nM range (13-15). Based on the results of studies by Chapuy *et al.* (16) and Ooms *et al.* (17), a 25(OH)D value of 30 nM as cut-off point for diagnosis of hypovitaminosis D was adopted for our present considerations.

It is important to note that hypovitaminosis D is not only frequently observed in housebound or hospitalized elderly people (16, 18-20), but is also a common phenomenon in the

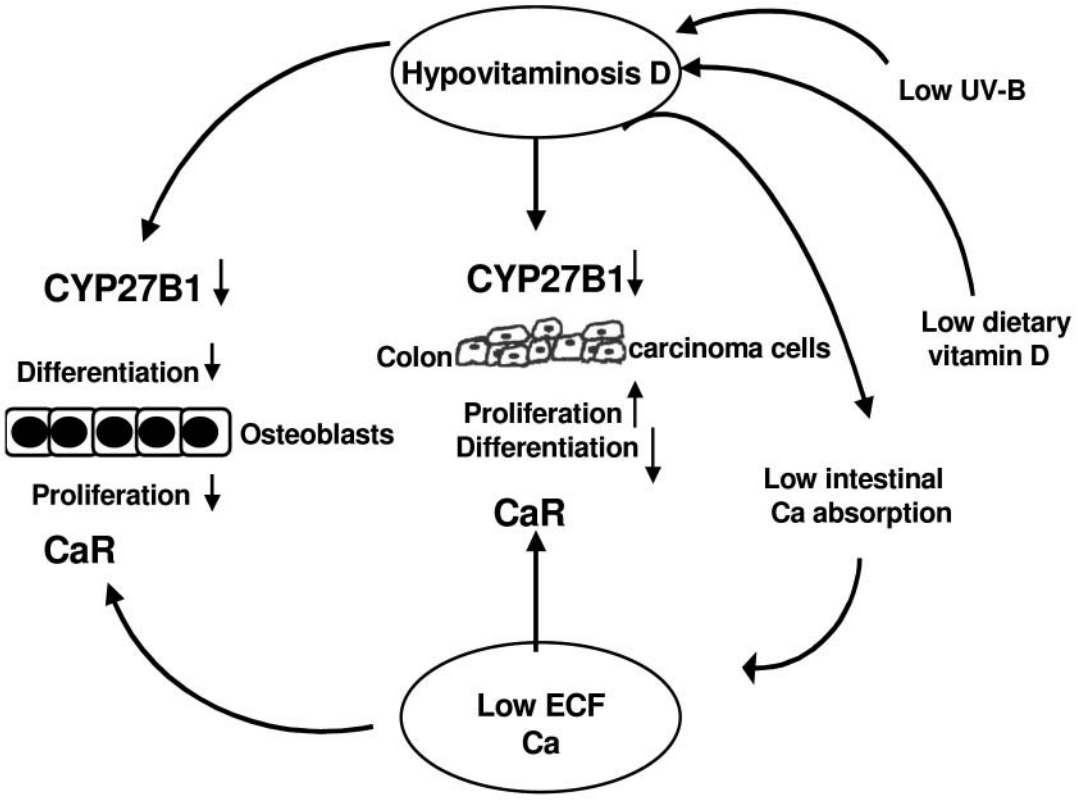

Figure 1. *Hypovitaminosis D and cell type-specific changes in proliferation and differentiation (CYP27B1, 25-hydroxyvitamin D-1α-hydroxylase; CaR, calcium-sensing receptor; ECF, extracellular fluid) (for details, see. (12)).*

free living younger population. For example, our large multi-center study on the calcium, vitamin D and bone status of a healthy adult Austrian population (21, 22) showed that 26% of all study participants had 25-(OH)D levels below 30 nM and thus presented with hypovitaminosis D.

Vitamin D insufficiency: differential effects on renal and extra-renal 1,25-$(OH)_2D_3$ synthesis. Under normal circumstances, renal CYP27B1 activity and production of 1,25-$(OH)_2D_3$ is tightly regulated by serum Ca^{++} and parathyroid hormone (PTH), as well as by feed-back inhibition from 1,25-$(OH)_2D_3$, as illustrated in Figure 2 (for details, see (23)). Therefore, the circulating 1,25-$(OH)_2D_3$ concentration is constant over a wide range of 25-(OH)D serum levels. However, this is not valid for extra-renal synthesis of 1,25-$(OH)_2D_3$, since, for example, CYP27B1 expression in intestinal epithelial cells (3) is considered to be insensitive to PTH and extracellular Ca^{++} or, as in macrophages or endothelial cells, is subject to modulation primarily by pro-inflammatory cytokines, such as interferon-γ (IFN-γ) (1, 10, 24). At extra-renal sites, the ambient 25-$(OH)D_3$ level becomes absolutely rate-limiting for intracellular synthesis of 1,25-$(OH)_2D_3$ (25). Therefore, hypovitaminosis D could create a situation in which locally produced 1,25-$(OH)_2D_3$ falls below a level that is critical for the maintenance of autocrine/paracrine regulation of cellular growth and function at sites of extrarenal CYP27B1 activity (*cf.* (26)).

Vitamin D Insufficiency and Pathogenesis of Chronic Diseases

Colorectal cancer

a) Epidemiological studies: A large body of evidence has accumulated over the years that an increased risk of colorectal cancer is associated with vitamin D insufficiency, *i.e.* low circulating 25-(OH)D (27-31), whereas no correlation was found between tumor incidence and serum 1,25-$(OH)_2D_3$ (32). As early as 1980, Garland *et al.* (27) had proposed that vitamin D may be a protective factor against colorectal cancer. They based this hypothesis on the observation that colon cancer mortality in the USA was highest in regions where the population was least exposed to solar radiation. Serum levels of 25-$(OH)D_3$ are a direct reflection of sunlight exposure, of the use of sun blocks and of skin pigmentation since, under normal circumstances, little vitamin D is obtained from nutritional sources (28) and, therefore, the

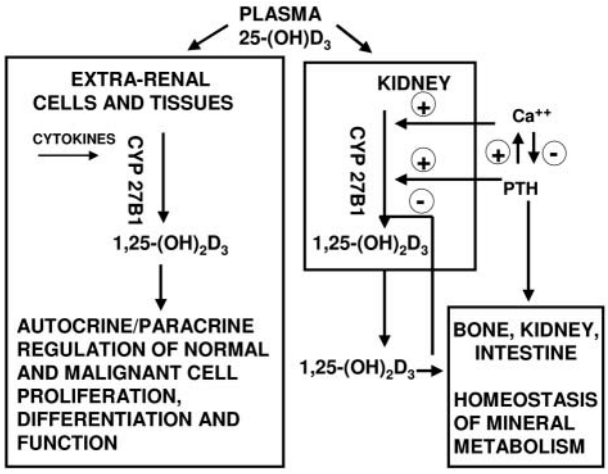

Figure 2. *Differential regulation of renal and extra-renal 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) activity.*

preventive effect of vitamin D consumption on colorectal cancer is modest (for review, (33)). Consequently, the impact of low sunshine exposure on vitamin D and on colorectal cancer incidence becomes that much greater. For example, in an ecological study of cancer mortality rates in the U.S. with respect to exposure to solar radiation, Grant (34) found a highly significant ($p<0.001$) inverse correlation between DNA-weighted UV-B radiation (280-315 nm) and the incidence of colorectal cancer. This association persisted even after additional ecological risk factors (smoking, dietary factors, urban residence, poverty *etc.*) were included in the analysis (35). Premature mortality due to insufficient UV-B exposure among white Americans was estimated at 12% of the total number of deaths from colon cancer (34).

b) The vitamin D endocrine system of the gut and control of cell growth. The gut apparently harbors a local vitamin D endocrine system. This is made up from epithelial and immune cells of the gut mucosa, which are not only targets for vitamin D receptor (VDR)-mediated 1,25-$(OH)_2D_3$ action, but also constitute a site of extra-renal vitamin D metabolism, because they express the 25-(OH)-D-1α-hydroxylase (CYP27B1) and the 25-(OH)-D-24-hydroxylase (CYP24) (see *e.g.*, (36)), the enzymes responsible for synthesis as well as degradation of 1,25-$(OH)_2D_3$. Our hypothesis is that the intestinal endocrine vitamin D system plays a critical role in maintaining the normal growth of epithelial cells (37). The obvious significant association between hypovitaminosis D and incidence of human colon cancer can be explained on a molecular and cellular basis by results of studies from our laboratory. First, it was observed that 1,25-$(OH)_2D_3$ functioned as a growth regulatory factor for human colon carcinoma cells (38-41); in post-confluent Caco-2 cell cultures, an ambient concentration of 10^{-11} M 1,25-$(OH)_2D_3$ was necessary to maintain the rate of cell division at the control level. An increase to 10^{-8} M brought about a 50% inhibition of cell proliferation, whereas reduction to 10^{-12} M stimulated the rate of cell division by up to 100% (38). Second, ours was the first group to show that normal and neoplastic human colonocytes expressed *CYP27B1* and were thus able to convert 25-$(OH)D_3$ into 1,25-$(OH)_2D_3$ (3, 4, 42). For these reasons, we put forward the notion that in hypovitaminosis D, *i.e.,* at low circulating 25-$(OH)D_3$, the rate of 1,25-$(OH)_2D_3$ production in the large intestinal mucosa be insufficient to achieve the tissue concentration necessary to maintain cellular homeostasis of the gut mucosa and, consequently, could facilitate neoplastic cell growth.

Other malignancies: Considering the importance of extra-renal production of 1,25-$(OH)_2D_3$ for control of cell proliferation, it would be expected that vitamin D insufficiency would also increase the risk of neoplastic transformation in the many cell types expressing *CYP27B1* (43-45). In fact, studies by Grant (34, 35, 46) as well as by Freedman *et al.* (47) on cancer mortality rates in the US and Europe, using latitude or DNA-weighted solar UV-B exposure as surrogate end-points for photoproduction of vitamin D_3 in the skin, found a highly significant association with the incidence of breast, esophagus, stomach, pancreas, bladder, ovary, uterus, prostate and non-melanoma skin cancer as well as non-Hodgkin lymphoma.

Osteoporosis. Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture (48). Underlying the accelerated bone loss, which is a hallmark of the disease, frequently is a low rate of bone formation. As shown by Owen *et al.* (49), 1,25-$(OH)_2D_3$ coordinates the sequence of osteoblast differentiation by specific VDR-mediated effects on the temporal gene expression and synthesis of several osteoblast proteins and is, therefore, indispensable for mineralized matrix maturation and bone formation. It is conceivable therefore, that low 25-(OH)D levels in vitamin D insufficiency, by limiting CYP27B1 activity in osteoblasts (6), have negative effects on bone formation and, consequently, increase the risk of osteoporosis. This notion is supported by observations in elderly people, who are at high risk for osteoporotic bone fractures and who typically present with low circulating 25-(OH)D, but normal 1,25$(OH)_2D_3$. Correction of vitamin D insufficiency by daily vitamin D_3 supplements was associated with a significant risk reduction for non-vertebral fractures (50).

Autoimmune diseases. Recent epidemiological studies indicate that a compromised vitamin D status in humans increases the risk of Th-1 cytokine-mediated autoimmune diseases, such as

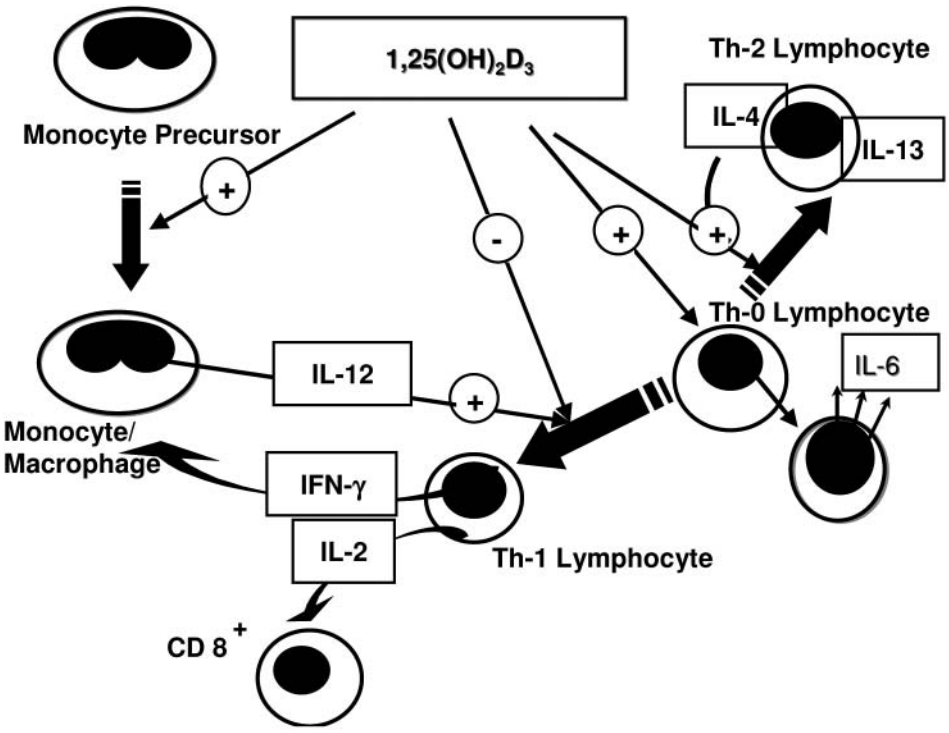

Figure 3. *1,25-Dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) and differentiation of immune cells (for details, see. (64, 66, 67)).*

inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematodes, multiple sclerosis as well as type I diabetes mellitus (51-58). In animal models of these diseases, administration of 1,25-$(OH)_2D_3$ has been shown to prevent the development, or at least to ameliorate the symptoms of a chronic inflammatory autoimmune reaction (59-63). The preventive effect of 1,25$(OH)_2D_3$ on chronic inflammatory and autoimmune diseases can be explained by findings that *in vitro* the steroid inhibits the release of pro-inflammatory cytokines, such as IL-2 and IFN-γ, from Th-1 lymphocyte (for review, (64); cf. Figure 3). It has to be taken into consideration that in lymphoid tissue, antigen-presenting cells, *i.e.*, macrophages and dendritic cells, are endowed with CYP27B1 activity (1, 2), so that they can use ambient 25-$(OH)D_3$ for conversion into the active vitamin D metabolite. This implies that vitamin D insufficiency, *i.e.,* low 25-$(OH)D_3$, is not only associated with impaired macrophage activation and function (65), but also that the local concentration of 1,25-$(OH)_2D_3$ in lymphoid tissue can be too low to effectively suppress a Th-1-type immune response.

Extensive evaluation of direct effects of 1,25-$(OH)_2D_3$ on human $CD4^+$ and $CD8^+$ T lymphocytes in our laboratory (66, 67) revealed that 1,25-$(OH)_2D_3$ had little effect on the constitutive expressions of Th1 and of Th-2 cytokines. Importantly, however, the steroid significantly inhibited IL-12-induced production of IFN-γ and IL-2 and, when Th-2 differentiation was induced by IL-4, significantly expanded the percentages of $IL\text{-}4^+$ and $IL\text{-}13^+$ cells. It must be emphasized that the predominant effect of 1,25-$(OH)_2D_3$ on T lymphocytes is the induction of separate $CD4^+$ and $CD8^+$ subpopulations with almost exclusive expression of IL-6 (Figure 3). This is an important facet of the immune modulating action of 1,25-$(OH)_2D_3$, because IL-6 may act in parallel with the steroid in modulation of T helper effector cell functions resulting in predominance of Th-2 over Th-1 responses. Consequently, vitamin D must be considered a useful agent for prevention and therapy of Th-1-mediated autoimmune diseases.

Inflammatory bowel disease. Although Crohn's disease and ulcerative colitis are two clinically distinct forms of inflammatory bowel disease (IBD), they have some basic pathogenetic features in common, such as an aberrant local immune reaction, *i.e.*, an excessive Th/Tc1 response to luminal, mainly bacterial antigens in genetically predisposed individuals (68, 69). In addition, evidence is accumulating linking the pathogenesis of IBD to a number of environmental factors (69), including vitamin D insufficiency (52, 53). Strong support for the assumption that a compromised vitamin D status plays a role in the pathogenesis of IBD also comes from studies with an animal model of Th-1 mediated colitis (59, 62).

It should be assumed that, as a consequence of hypovitaminosis D, local production of 1,25-$(OH)_2D_3$ by mucosal epithelial cells as well as by macrophages and dendritic cells (1, 2) in the vitamin D endocrine system of the gut falls below a level that is critical for the suppression of enhanced Th1 cell reactions that arise from challenge with luminal antigens. By the same token, vitamin D insufficiency could aid in further propagating the aberrant immune response, which causes the kind of inflammatory lesions that are typically associated with chronic enterocolitis (68). In addition, attenuation of the antiproliferative action of 1,25-$(OH)_2D_3$ is associated with site-specific hyperproliferation in the colon (37) and can be seen as a predisposing condition for colitis-associated cancer (70).

Multiple sclerosis. Multiple sclerosis is an autoimmune disease in which overreaction of the Th-1 lymphocyte system to an, as yet unidentified, antigenic stimulus leads to an immune attack on the central nervous system. An association between hypovitaminosis D due to low sunlight exposure and incidence of multiple sclerosis was first recognized by Goldberg (71, 72) 30 years ago. Since then, this notion has been supported by data from additional epidemiological as well as experimental animal studies (for review, (57, 58)). From studies on experimental autoimmune encephalomyelitis, which is an animal model of the human disease, it became clear that inefficient suppression by 1,25-$(OH)_2D_3$ of Th-1 lymphocyte function was a major pathogenetic factor of the disease (60, 73).

Diabetes mellitus type I. There is substantial evidence from studies with non-obese diabetic mice, that vitamin D deficiency in early life accelerates the appearance of the symptoms of autoimmune diabetes mellitus (74) and that, conversely, 1,25-$(OH)_2D_3$ can prevent the development of the disease (*cf. e.g.*, (75, 76)). Recently, Hypponen *et al.* (54) reported the results of a large birth-cohort study highlighting the importance of vitamin D supplementation for the prevention of diabetes mellitus type I in children. Their data clearly showed that regular vitamin D intake as compared to no supplementation during the first year of life was associated with 88% risk reduction of type I diabetes mellitus in later life. Even children who received vitamin D irregularly had a 84% lower risk than those with no supplementation. It must be emphasized that this study is, to date, the first report that showed the efficacy of vitamin D substitution or supplementation in the prevention of an autoimmune disease in humans.

Vitamin D and Calcium Interactions

Hypovitaminosis D is frequently associated with intestinal calcium malabsorption and, consequently, with a reduction of extracellular fluid calcium concentrations (Figure 1). Many cell types are endowed with a plasma membrane-bound extracellular calcium-sensing receptor (CaR) (77, 78), which transduces even small variations in extracellular calcium into cell-specific intracellular signaling pathways. Frequently, signaling from the CaR causes changes in cellular proliferation or differentiation in the same direction as does the VDR-mediated action of 1,25-$(OH)_2D_3$ (Figure 1). Therefore, vitamin D and calcium insufficiency contribute to the development of chronic diseases though by different mechanisms. The calcium and vitamin D status appear mainly to act together, such as in the pathogenesis of osteoporosis, colorectal and breast cancer and probably also of autoimmune diabetes type I and multiple sclerosis (for review, (12)). With respect to osteoporosis, it is commonly accepted that combined vitamin D and calcium supplementation is the basis of any therapeutic measures (50, 79). Grau *et al.* (80), in their study on the effect of vitamin D and calcium supplementation on recurrence of colorectal adenomas, found that calcium supplementation was effective only in patients with normal 25-(OH)D values. Conversely, high 25-(OH)D levels were associated with a reduced risk of adenoma recurrence only among subjects receiving calcium supplements. Synergistic actions of calcium and vitamin D are probably the reason why high intake of low-fat dairy products is associated with a reduced risk of breast cancer in pre-menopausal women (81). Finally, results from studies in animal models of human autoimmune diseases indicated that calcium supplementation was necessary to optimize the therapeutic effect of vitamin D (73, 82). Therefore, correction of both a nutritional calcium deficit and a compromised vitamin D status is required to prevent the development of some of the most frequent chronic diseases. The fact that almost one quarter of the adult population is both vitamin D and calcium insufficient (12) poses a special challenge for preventive medicine and public health policies alike.

References

1 Adams JS, Singer FR, Gacad MA, Sharma OP, Hayes MJ, Vouros P and Holick MF: Isolation and structural identification of 1,25-dihydroxyvitamin D_3 produced by cultured alveolar macrophages in sarcoidosis. J Clin Endocrinol Metab *60*: 960-966, 1985.

2 Hewison M, Freeman L, Hughes SV, Evans KN, Bland R, Eliopoulos AG, Kilby MD, Moss PAH and Chakraverty R: Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells. J Immunol *170*: 5382-5390, 2003.

3 Cross HS, Peterlik M, Reddy GS and Schuster I: Vitamin D metabolism in human colon adenocarcinoma-derived Caco-2 cells: expression of 25-hydroxyvitamin D_3-1alpha-hydroxylase activity and regulation of side-chain metabolism. J Steroid Biochem Mol Biol *62*: 21-28, 1997.

4 Bareis P, Bises G, Bischof MG, Cross HS and Peterlik M: 25-hydroxy-vitamin D metabolism in human colon cancer cells during tumor progression. Biochem Biophys Res Commun *285*: 1012-1017, 2001.

5 Cross HS, Bareis P, Hofer H, Bischof MG, Bajna E, Kriwanek S, Bonner E and Peterlik M: 25-Hydroxyvitamin D-3-1 alpha-hydroxylase and vitamin D receptor gene expression in human colonic mucosa is elevated during early cancerogenesis. Steroids *66*: 287-292, 2001.

6 Siu-Caldera ML, Zou L, Ehrlich MG, Schwartz ER, Ishizuka S and Reddy GS: Human osteoblasts in culture metabolize both 1 alpha, 25-dihydroxyvitamin D_3 and its precursor 25-hydroxyvitamin D_3 into their respective lactones. Endocrinology *136*: 4195-4203, 1995.

7 Inaba M, Yukioka K, Furumitsu Y, Murano M, Goto H, Nishizawa Y and Morii H: Positive correlation between levels of IL-1 or IL-2 and $1,25(OH)_2D$/25-OH-D ratio in synovial fluid of patients with rheumatoid arthritis. Life Sci *61*: 977-985, 1997.

8 Pillai S, Bikle DD and Elias PM: 1,25-Dihydroxyvitamin D production and receptor binding in human keratinocytes varies with differentiation. J Biol Chem *263*: 5390-5395, 1988.

9 Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM and Hewison M: Extrarenal expression of 25-hydroxyvitamin D(3)-1 alpha-hydroxylase. J Clin Endocrinol Metab *86*: 888-894, 2001.

10 Zehnder D, Bland R, Chana RS, Wheeler DC, Howie AJ, Williams MC, Stewart PM and Hewison M: Synthesis of 1,25-dihydroxyvitamin D(3) by human endothelial cells is regulated by inflammatory cytokines: a novel autocrine determinant of vascular cell adhesion. J Am Soc Nephrol *13*: 621-629, 2002.

11 Zittermann A: Vitamin D in preventive medicine: are we ignoring the evidence? Br J Nutr *89*: 552-572, 2003.

12 Peterlik M and Cross HS: Vitamin D and calcium deficits predispose for multiple chronic diseases. Eur J Clin Invest *35*: 290-304, 2005.

13 Vieth R: Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr *69*: 842-856, 1999.

14 Vieth R: Why the optimal requirement for Vitamin D_3 is probably much higher than what is officially recommended for adults. J Steroid Biochem Mol Biol *89-90*: 575-579, 2004.

15 Whiting SJ and Calvo MS: Dietary recommendations for vitamin D: a critical need for functional end points to establish an estimated average requirement. J Nutr *135*: 304-309, 2005.

16 Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S and Meunier PJ: Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int *7*: 439-443, 1997.

17 Ooms ME, Lips P, Roos JC, van der Vijgh WJ, Popp-Snijders C, Bezemer PD and Bouter LM: Vitamin D status and sex hormone binding globulin: determinants of bone turnover and bone mineral density in elderly women. J Bone Miner Res *10*: 1177-1184, 1995.

18 Lamberg-Allardt CJ, Outila TA, Karkkainen MU, Rita HJ and Valsta LM: Vitamin D deficiency and bone health in healthy adults in Finland: could this be a concern in other parts of Europe? J Bone Miner Res *16*: 2066-2073, 2001.

19 Scharla SH: Prevalence of subclinical vitamin D deficiency in different European countries. Osteoporos Int *8 Suppl 2*: S7-12, 1998.

20 Dawson-Hughes B, Harris SS and Dallal GE: Plasma calcidiol, season, and serum parathyroid hormone concentrations in healthy elderly men and women. Am J Clin Nutr *65*: 67-71, 1997.

21 Kudlacek S, Schneider B, Peterlik M, Leb G, Klaushofer K, Weber K, Woloszczuk W and Willvonseder R: Normative data of bone mineral density in an unselected adult Austrian population. Eur J Clin Invest *33*: 332-339, 2003.

22 Kudlacek S, Schneider B, Peterlik M, Leb G, Klaushofer K, Weber K, Woloszczuk W and Willvonseder R: Assessment of vitamin D and calcium status in healthy adult Austrians. Eur J Clin Invest *33*: 323-331, 2003.

23 Broadus A: E. Mineral Balance and Homeostasis. *In*: Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. Favus MJ (ed.). Washington, DC: American Society for Bone and Mineral Research, pp. 105-111, 2003.

24 Overbergh L, Decallonne B, Valckx D, Verstuyf A, Depovere J, Laureys J, Rutgeerts O, Saint-Arnaud R, Bouillon R and Mathieu C: Identification and immune regulation of 25-hydroxyvitamin D-1-alpha-hydroxylase in murine macrophages. Clin Exp Immunol *120*: 139-146, 2000.

25 Vieth R: The pharmacology of vitamin D, including fortification strategies. *In*: Vitamin D. Feldman D, Pike WJ, Glorieux FH (eds.). Amsterdam: Elsevier, pp. 995-1015, 2005.

26 Tuohimaa P, Tenkanen L, Ahonen M, Lumme S, Jellum E, Hallmans G, Stattin P, Harvei S, Hakulinen T, Luostarinen T, Dillner J, Lehtinen M and Hakama M: Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. Int J Cancer *108*: 104-108, 2004.

27 Garland CF and Garland FC: Do sunlight and vitamin D reduce the likelihood of colon cancer? Int J Epidemiol *9*: 227-231, 1980.

28 Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, Allen C, Doughertly C, Gunter EW and Bowman BA: Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. Am J Clin Nutr *76*: 187-192, 2002.

29 Garland CF, Comstock GW, Garland FC, Helsing KJ, Shaw EK and Gorham ED: Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. Lancet *2*: 1176-1178, 1989.

30 Holt PR, Arber N, Halmos B, Forde K, Kissileff H, McGlynn KA, Moss SF, Kurihara N, Fan K, Yang K and Lipkin M: Colonic epithelial cell proliferation decreases with increasing levels of serum 25-hydroxy vitamin D. Cancer Epidemiol Biomarkers Prev *11*: 113-119, 2002.

31 Feskanich D, Ma J, Fuchs CS, Kirkner GJ, Hankinson SE, Hollis BW and Giovannucci EL: Plasma vitamin D metabolites and risk of colorectal cancer in women. Cancer Epidemiol Biomarkers Prev *13*: 1502-1508, 2004.

32 Tangrea J, Helzlsouer K, Pietinen P, Taylor P, Hollis B, Virtamo J and Albanes D: Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. Cancer Causes Control *8*: 615-625, 1997.

33 Grant WB and Garland CF: A critical review of studies on vitamin D in relation to colorectal cancer. Nutr Cancer *48*: 115-123, 2004.

34 Grant WB: An estimate of premature cancer mortality in the U.S. due to inadequate doses of solar ultraviolet-B radiation. Cancer *94*: 1867-1875, 2002.

35 Grant WB: Ecologic studies of solar UV-B radiation and cancer mortality rates. Rec Results Cancer Res *164*: 371-377, 2003.

36 Bareis P, Bises G, Bischof MG, Cross HS and Peterlik M: 25-hydroxyvitamin D metabolism in human colon cancer cells during tumor progression. Biochem Biophys Res Commun *285*: 1012-1017, 2001.

37 Kallay E, Pietschmann P, Toyokuni S, Bajna E, Hahn P, Mazzucco K, Bieglmayer C, Kato S and Cross HS: Characterization of a vitamin D receptor knockout mouse as a model of colorectal hyperproliferation and DNA damage. Carcinogenesis *22*: 1429-1435, 2001.

38 Cross HS, Pavelka M, Slavik J and Peterlik M: Growth control of human colon cancer cells by vitamin D and calcium *in vitro*. J Natl Cancer Inst *84*: 1355-1357, 1992.

39 Cross HS, Hulla W, Tong WM and Peterlik M: Growth regulation of human colon adenocarcinoma-derived cells by calcium, vitamin D and epidermal growth factor. J Nutr *125(7 Suppl)*: 2004S-2008S, 1995.

40 Hulla W, Kallay E, Krugluger W, Peterlik M and Cross HS: Growth control of human colon-adenocarcinoma-derived Caco-2 cells by vitamin-D compounds and extracellular calcium *in vitro*: relation to c-myc-oncogene and vitamin-D-receptor expression. Int J Cancer *62*: 711-716, 1995.

41 Tong WM, Hofer H, Ellinger A, Peterlik M and Cross HS: Mechanism of antimitogenic action of vitamin D in human colon carcinoma cells: relevance for suppression of epidermal growth factor-stimulated cell growth. Oncol Res *11*: 77-84, 1999.

42 Bises G, Kallay E, Weiland T, Wrba F, Wenzl E, Bonner E, Kriwanek S, Obrist P and Cross HS: 25-hydroxyvitamin D_3-1alpha-hydroxylase expression in normal and malignant human colon. J Histochem Cytochem *52*: 985-989, 2004.

43 Friedrich M, Rafi L, Mitschele T, Tilgen W, Schmidt W and Reichrath J: Analysis of the vitamin D system in cervical carcinomas, breast cancer and ovarian cancer. Rec Results Cancer Res *164*: 239-246, 2003.

44 Schwartz GG, Whitlatch LW, Chen TC, Lokeshwar BL and Holick MF: Human prostate cells synthesize 1,25-dihydroxyvitamin D_3 from 25-hydroxyvitamin D3. Cancer Epidemiol Biomarkers Prev *7*: 391-395, 1998.

45 Schwartz GG, Eads D, Rao A, Cramer SD, Willingham MC, Chen TC, Jamieson DP, Wang L, Burnstein KL, Holick MF and Koumenis C: Pancreatic cancer cells express 25-hydroxyvitamin D-1 alpha-hydroxylase and their proliferation is inhibited by the prohormone 25-hydroxyvitamin D_3. Carcinogenesis *25*: 1015-1026, 2004.

46 Grant WB: An ecologic study of dietary and solar ultraviolet-B links to breast carcinoma mortality rates. Cancer *94*: 272-281, 2002.

47 Freedman DM, Dosemeci M and McGlynn K: Sunlight and mortality from breast, ovarian, colon, prostate, and non-melanoma skin cancer: a composite death certificate based case-control study. Occup Environ Med *59*: 257-262, 2002.

48 Anonymous: Osteoporosis prevention, diagnosis, and therapy. JAMA *285*: 785-795, 2001.

49 Owen TA, Aronow MS, Barone LM, Bettencourt B, Stein GS and Lian JB: Pleiotropic effects of vitamin D on osteoblast gene expression are related to the proliferative and differentiated state of the bone cell phenotype: dependency upon basal levels of gene expression, duration of exposure, and bone matrix competency in normal rat osteoblast cultures. Endocrinology *128*: 1496-1504, 1991.

50 Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD and Meunier PJ: Vitamin D_3 and calcium to prevent hip fractures in the elderly women. N Engl J Med *327*: 1637-1642, 1992.

51 Grant WB: Solar UV-B radiation is linked to the geographic variation of mortality from systemic lupus erythematosus in the USA. Lupus *13*: 281-282, 2004.

52 Sonnenberg A, McCarty DJ and Jacobsen SJ: Geographic variation of inflammatory bowel disease within the United States. Gastroenterology *100*: 143-149, 1991.

53 Podolsky DK: Inflammatory bowel disease. N Engl J Med *325*: 928-1016, 1991.

54 Hypponen E, Laara E, Reunanen A, Jarvelin MR and Virtanen SM: Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet *358*: 1500-1503, 2001.

55 Merlino L, Curtis J, Mikuls TR, Cerhan JR, Criswell LA and Saag KG: Vitamin D intake is inversely associated with rheumatoid arthritis. Results from the Iowa Women's Health Study. Arthr Rheumat *50*: 72-77, 2004.

56 Ponsonby AL, McMichael A and van der Mei I: Ultraviolet radiation and autoimmune disease: insights from epidemiological research. Toxicology *181-182*: 71-78, 2002.

57 Embry A: Vitamin D supplementation in the fight against multiple sclerosis. J Orthomol Med *19*: 27-38, 2004.

58 Embry A: The multiple factors of multiple sclerosis. J Nutr Environm Med *14*: 1-11, 2004.

59 Froicu M, Weaver V, Wynn TA, McDowell MA, Welsh JE and Cantorna MT: A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. Mol Endocrinol *17*: 2386-2392, 2003.

60 Cantorna MT, Hayes CE and DeLuca HF: 1,25-Dihydroxyvitamin D_3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. Proc Natl Acad Sci USA *93*: 7861-7864, 1996.

61 Cantorna MT, Hayes CE and DeLuca HF: 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. J Nutr *128*: 68-72, 1998.

62 Cantorna MT, Munsick C, Bemiss C and Mahon BD: 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. J Nutr *130*: 2648-2652, 2000.

63 Branisteanu DD, Waer M, Sobis H, Marcelis S, Vandeputte M and Bouillon R: Prevention of murine experimental allergic encephalomyelitis: cooperative effects of cyclosporine and 1 alpha, 25-$(OH)_2D_3$. J Neuroimmunol *61(2)*: 151-160, 1995.

64 Hayes CE, Nashold FE, Spach KM and Pedersen LB: The immunological functions of the vitamin D endocrine system. Cell Mol Biol (Noisy-le-grand) *49*: 277-300, 2003.

65 Gavison R and Bar-Shavit Z: Impaired macrophage activation in vitamin D_3 deficiency: differential *in vitro* effects of 1,25-dihydroxyvitamin D_3 on mouse peritoneal macrophage functions. J Immunol *143*: 3686-3690, 1989.

66 Thien R, Baier K, Pietschmann P, Peterlik M and Willheim M: Interactions of 1 alpha,25-dihydroxyvitamin D_3 with IL-12 and IL-4 on cytokine expression of human T lymphocytes. J Allergy Clin Immunol *116*: 683-689, 2005.

67 Willheim M, Thien R, Schrattbauer K, Bajna E, Holub M, Gruber R, Baier K, Pietschmann P, Reinisch W, Scheiner O and Peterlik M: Regulatory effects of 1alpha,25-dihydroxyvitamin D_3 on the cytokine production of human peripheral blood lymphocytes. J Clin Endocrinol Metab *84*: 3739-3744, 1999.

68 Targan SR and Murphy LK: Clarifying the causes of Crohn's. Nat Med *1*: 1241-1243, 1995.

69 Farrell RJ and Peppercorn MA: Ulcerative colitis. Lancet *359*: 331-340, 2002.

70 Itzkowitz S: Colon carcinogenesis in inflammatory bowel disease: applying molecular genetics to clinical practice. J Clin Gastroenterol *36 (5 Suppl)*: S70-74 2003 .

71 Goldberg P: Multiple sclerosis; vitamin D and calcium as environmental determinants of prevalence. Part 1: Sunlight, dietary facrors and epidemiology. Int J Environ Studies *6*: 19-27, 1974.

72 Goldberg P: Multiple sclerosis; vitamin D and calcium as environmental determinants of prevalence. Part 2: Biochemical and genetic factors. Int J Environ Studies *6*: 121-129, 1974.

73 Cantorna MT, Humpal-Winter J and DeLuca HF: Dietary calcium is a major factor in 1,25-dihydroxycholecalciferol suppression of experimental autoimmune encephalomyelitis in mice. J Nutr *129*: 1966-1971, 1999.

74 Giulietti A, Gysemans C, Stoffels K, Van Etten E, Decallonne B, Overbergh L, Bouillon R and Mathieu C: Vitamin D deficiency in early life accelerates type 1 diabetes in non-obese diabetic mice. Diabetologia *47*: 451-462, 2004.

75 Mathieu C, Waer M, Laureys J, Rutgeerts O and Bouillon R: Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D_3. Diabetologia *37*: 552-558, 1994.

76 Casteels K, Bouillon R, Waer M and Mathieu C: Immunomodulatory effects of 1,25-dihydroxyvitamin D_3. Curr Opin Nephrol Hypertens *4*: 313-318, 1995.

77 Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J and Hebert SC: Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. Nature *366*: 575-580, 1993.

78 Chattopadhyay N and Brown EM: Calcium-sensing Receptor. Boston: Kluwer Academic Publishers, 2003.

79 Larsen ER, Mosekilde L and Foldspang A: Vitamin D and calcium supplementation prevents osteoporotic fractures in elderly community dwelling residents: a pragmatic population-based 3-year intervention study. J Bone Miner Res *19*: 370-378, 2004.

80 Grau MV, Baron JA, Sandler RS, Haile RW, Beach ML, Church TR and Heber D: Vitamin D, calcium supplementation, and colorectal adenomas: results of a randomized trial. J Natl Cancer Inst *95*: 1765-1771, 2003.

81 Shin MH, Holmes MD, Hankinson SE, Wu K, Colditz GA and Willett WC: Intake of dairy products, calcium, and vitamin D and risk of breast cancer. J Natl Cancer Inst *94*: 1301-1311, 2002.

82 Mathieu C, Van Etten E, Gysemans C, Decallonne B, Kato S, Laureys J, Depovere J, Valckx D, Verstuyf A and Bouillon R: *In vitro* and *in vivo* analysis of the immune system of vitamin D receptor knockout mice. J Bone Miner Res *16*: 2057-2065, 2001.

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