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Review

Vitamin D physiology

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Abstract

Vitamin D3 is synthesized in the skin during summer under the influence of ultraviolet light of the sun, or it is obtained from food, especially fatty fish. After hydroxylation in the liver into 25-hydroxyvitamin D (25(OH)D) and kidney into 1,25-dihydroxyvitamin D (1,25(OH)2D), the active metabolite can enter the cell, bind to the vitamin D-receptor and subsequently to a responsive gene such as that of calcium binding protein. After transcription and translation the protein is formed, e.g. osteocalcin or calcium binding protein. The calcium binding protein mediates calcium absorption from the gut. The production of 1,25(OH)2D is stimulated by parathyroid hormone (PTH) and decreased by calcium. Risk factors for vitamin D deficiency are premature birth, skin pigmentation, low sunshine exposure, obesity, malabsorption and advanced age. Risk groups are immigrants and the elderly. Vitamin D status is dependent upon sunshine exposure but within Europe, serum 25(OH)D levels are higher in Northern than in Southern European countries. Severe vitamin D deficiency causes rickets or osteomalacia, where the new bone, the osteoid, is not mineralized. Less severe vitamin D deficiency causes an increase of serum PTH leading to bone resorption, osteoporosis and fractures. A negative relationship exists between serum 25(OH)D and serum PTH. The threshold of serum 25(OH)D, where serum PTH starts to rise is about 75 nmol/l according to most surveys. Vitamin D supplementation to vitamin D-deficient elderly suppresses serum PTH, increases bone mineral density and may decrease fracture incidence especially in nursing home residents. The effects of 1,25(OH)2D and the vitamin D receptor have been investigated in patients with genetic defects of vitamin D metabolism and in knock-out mouse models. These experiments have demonstrated that for active calcium absorption, longitudinal bone growth and the activity of osteoblasts and osteoclasts both 1,25(OH)2D and the vitamin D receptor are essential. On the other side, bone mineralization can occur by high ambient calcium concentration, so by high doses of oral calcium or calcium infusion. The active metabolite 1,25(OH)2D has its effects through the vitamin D receptor leading to gene expression, e.g. the calcium binding protein or osteocalcin or through a plasma membrane receptor and second messengers such as cyclic AMP. The latter responses are very rapid and include the effects on the pancreas, vascular smooth muscle and monocytes. Muscle cells contain vitamin D receptor and several studies have demonstrated that serum 25(OH)D is related to physical performance. The active metabolite 1,25(OH)2D has an antiproliferative effect and downregulates inflammatory markers. Extrarenal synthesis of 1,25(OH)2D occurs under the influence of cytokines and is important for the paracrine regulation of cell differentiation and function. This may explain that vitamin D deficiency can play a role in the pathogenesis of auto-immune diseases such as multiple sclerosis and diabetes type 1, and cancer. In conclusion, the active metabolite 1,25(OH)2D has pleiotropic effects through the vitamin D receptor and vitamin D responsive elements of many genes and on the other side rapid non-genomic effects through a membrane receptor and second messengers. Active calcium absorption from the gut depends on adequate formation of 1,25(OH)2D and an intact vitamin D receptor. Bone

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mineralization mainly depends on ambient calcium concentration. Vitamin D metabolites may play a role in the prevention of auto-immune disease and cancer.

Keywords: Vitamin D deficiency; Bone mineralization; Auto-immune disease; Vitamin D: genomic and non-genomic effects; Vitamin D-dependent rickets; Vitamin D receptor

Contents

1. Introduction

The first clinical description of the vitamin D-related disease, rickets, was made by Glisson in 1651. He described children with rickets, as was called at that time the Morbus Anglorum, because it was quite common in England. The association between lack of sunshine and rickets was first recognized in the beginning of the 20th century and around 1920 healing of rickets with sunlight was reported (Holick, 1994). In this article, I will discuss vitamin D metabolism, calcium homeostasis, the effects of vitamin D on bone, genomic and non-genomic effects of vitamin D and finally extraskeletal effects on muscle, on the immune system, on cell differentiation and on the pancreas.

2. Physiology

The two main forms of vitamin D are: vitamin D3 or cholecalciferol, which is formed in the skin after exposure to sunlight or ultraviolet light, and ergocalciferol or vitamin D2 which is obtained by irradiation of plants or plant materials or foods. The differences are situated in the side chain. Vitamin D3 is synthesized in the skin during summer months or it is obtained from nutritional sources, especially fatty fish such as herring and mackerel. Limitations are age, a pigmented skin, sunscreen use and clothing. Vitamin D3 or cholecalciferol is hydroxylated in the liver into 25-hydroxyvitamin D3 (25(OH)D) and subsequently in the kidney into 1,25-dihydroxyvitamin D3 (1,25(OH)2D). This is the active metabolite, which stimulates the calcium absorption from the gut (Feldman et al., 2005). When 1,25(OH)2D is sufficiently available, 24,25-dihydroxyvitamin D (24,25(OH)2D) is formed in the kidney, which is further catabolized. The vitamin D metabolites are bound in the circulation to vitamin D binding protein which has a high affinity to 25(OH)D, 24,25(OH)2D and 1,25(OH)2D and has a high homology to albumin. The active metabolite 1,25(OH)2D enters the cell and binds to the vitamin D receptor. This complex forms a heterodimer with the retinoid receptor and binds to a vitamin D responsive element on a responsive gene, such as that of osteocalcin, calcium binding protein or 24-hydroxylase. This is followed by transcription and translation and proteins are formed such as the calcium binding protein or osteocalcin. The classic effect of 1,25(OH)2D on active calcium transport occurs in the intestinal cell. Calcium enters the cell through membrane proteins. In the intestinal cell, 1,25(OH)2D binds to the vitamin D receptor and the calcium binding protein is synthesized and this regulates the active transport through the cell. The calcium is transported to the extracellular fluid by an ATP-dependent mechanism. There also is passive transport through paracellular diffusion of calcium. The vitamin D-dependent calcium aborption has a maximum. The vitamin D-independent calcium absorption through passive diffusion does not have a maximum, but depends on the calcium gradient, this means on the calcium intake.

The 1,25(OH)2D has its effect on the classic target organs bone, intestine and kidney and stimulates calcium transport from these organs to the blood. The production of 1,25(OH)2D is stimulated by parathyroid hormone (PTH). There is a negative feedback through calcium which decreases PTH and a direct negative feedback from 1,25(OH)2D to PTH. The active metabolite 1,25(OH)2D also shows rapid actions through a membrane receptor.

3. Risk factors for vitamin D deficiency

Risk factors for vitamin D deficiency are premature and dysmature birth, pigmented skin, low sunshine exposure, obesity, malabsorption and advanced age as the aged skin produces much less vitamin D than the skin in younger people. Rickets was highly prevalent around 1900 in large cities. Nowadays in the Netherlands it is observed among immigrant children due to low sunshine exposure, skin pigmentation and diet. The prevalence of vitamin D deficiency is also high in elderly people compared with adults and especially in residents of homes for the elderly, nursing homes and patients with hip fracture (Lips, 2001). The prevalence of vitamin D deficiency is much higher in Europe than in Asia, Australia or the USA. Within Europe, serum 25(OH)D is positively related to latitude, contrary to what should be expected (Lips et al., 2001). The highest serum 25(OH)D levels were observed in Scandinavian countries and the lowest levels were found in Southern Europe. This may be due to high sun exposure, a light skin and multivitamin use in northern countries while shadow-seeking behaviour and a darker skin are more common in mediterranean countries. Vitamin D deficiency is very common in elderly people, especially in the institutionalized, with a prevalence up to more than 75% in nursing home residents (Holick, 1994). The prevalence of vitamin D insufficiency is also high in Afro-Americans, in which the highly pigmented skin makes the ultraviolet light much less efficacious (Holick, 1994). A high prevalence of vitamin D deficiency has been reported in non-western immigrants in the Netherlands (Grootjans-Geerts, 2001) and similar data have been obtained in the Middle East (Gannage-Yared et al., 2000), where life-style factors probably play a role.

4. Consequences of vitamin D deficiency

Severe vitamin D deficiency causes rickets or osteomalacia. In osteomalacia, most surfaces of trabecular and cortical bone are covered with thick osteoid seams. It is very different from osteoporosis where usually only small amounts of osteoid are visible. Vitamin D deficiency also causes higher secretion of PTH due to the low serum 1,25(OH)2D and low serum calcium, and this results in high bone turnover and increased bone resorption. This causes bone loss, mainly from cortical bone and this may contribute to the pathogenesis of osteoporosis. So, on one side severe vitamin D deficiency causes a mineralization problem and osteomalacia and on the other side the high PTH levels cause high bone turnover, bone resorption and osteoporosis and both mechanisms may lead to fractures, especially hip fractures (Lips, 2001). So, there is an inverse relationship between serum 25(OH)D and parathyroid hormone. This was investigated in the Longitudinal Aging Study Amsterdam in 1320 older men and women. Serum PTH decreases when serum 25(OH)D increases and serum PTH stabilizes when there is sufficient 25(OH)D. This plateau was reached around 75 nmol/l, a much higher level than was previously assumed (Lips et al., 2005). Similar data also have been reported from the USA and France. Vitamin D supplementation to vitamin D-deficient elderly results in an increase of serum 25(OH)D levels and decrease of serum PTH and an increase of bone mineral density. This increase may depend on variations in the vitamin D receptor DNA structure, so called polymorphisms. In the vitamin D study performed in Amsterdam, the increase of bone mineral density in the femoral neck with vitamin D supplementation depended on the vitamin D receptor genotype (Lips, 2001).

5. Genomic and non-genomic effects of 1,25(OH)2D

The main effect of 1,25(OH)2D is to increase the absorption of calcium from the gut. Does it also increase osteoid mineralization, stimulate osteoblast function and osteoclast function and suppress PTH secretion? These questions have been investigated in patients with genetic defects of vitamin D metabolism and in knock-out mouse models. In patients with vitamin D-dependent rickets type 1 or pseudovitamin D deficiency rickets

the 1 α-hydroxylase enzyme is not functioning, causing very low serum levels of 1,25(OH)2D. These patients may be treated with physiological doses of 1,25(OH)2D and this resulted in complete healing of the rickets (Feldman et al., 2005). Patients with vitamin D-dependent rickets type II or hereditary vitamin D resistant rickets do not have a functioning vitamin D receptor. These children with short stature and baldness have mutations in the zinc fingers of the VDR DNA binding domain. These patients can be treated with calcium infusions, leading to healing of the rickets (Feldman et al., 2005). Panda et al. did a series of elegant experiments with wild type mice, 1 α-hydroxylase knock-out mice, VDR knock-out mice and a combination of the two knock-outs (Panda et al., 2004). The four groups of mice were treated with either a high calcium diet or a rescue diet with very high calcium and lactose or with 1,25(OH)2D. In the 1 α-hydroxylase knock-out mouse, physiological doses of 1,25(OH)2D corrected longitudinal bone growth to normal size. The high calcium diet and the rescue diet could not correct longitudinal bone growth in the 1 α-hydroxylase knock-out. The longitudinal bone growth in the VDR knock-outs could not be corrected by diets or 1,25(OH)2D. However, the rickets could be completely cured with the rescue diet containing a very high calcium content and lactose. So, it appears that for the healing of rickets neither 1 α-hydroxylase nor the vitamin D receptor is necessary. On the other side, the rickets could not be healed by 1,25(OH)2D and calcium diet in the absence of the vitamin D receptor. In summary, these experiments showed that for calcium absorption, longitudinal bone growth, osteoblast activity and osteoclast activity, both 1,25(OH)2D and the vitamin D receptor are essential. Bone mineralization can occur by a high ambient calcium concentration so by a high dose of oral calcium or calcium infusion. Calcium can also very effectively suppress PTH secretion. To decrease the parathyroid size to normal gland size, calcium and 1,25(OH)2D are necessary.

The active metabolite 1,25(OH)2D can exert its function through the vitamin D receptor leading to gene expression, either upregulation or downregulation of gene products such as calcium binding protein or osteocalcin. This may take hours or days. On the other side, 1,25(OH)2D may work through a plasma membrane receptor and second messengers such as MAP kinase or cyclic AMP, and this may influence calcium channels (Feldman et al., 2005). The rapid responses through a second messenger include the effect on the pancreas β-cell, on vascular smooth muscle, on the intestine and on monocytes. The active metabolite 1,25(OH)2D stimulates calcium absorption, decreases PTH secretion, stimulates osteoclastic bone resorption, stimulates the osteoblasts, decreases the production of collagen type 1, influences muscular function, stimulates cell differentiation and the immune system and influences insulin secretion. Muscle cells contain vitamin D receptors and in the large population study NHANES III the walking test and the chair stand test were positively influenced (took less time) when serum 25(OH)D was higher. Muscle function was optimal with a 25(OH)D level higher than 60 nmol/l (Bischoff-Ferrari et al., 2004a). The same investigators studied muscle biopsies of 32 women by immunohistochemical staining of the vitamin D receptor and it was observed that the number of vitamin D receptors decreased with aging (Bischoff-Ferrari et al., 2004b). We have also studied vitamin D status and physical performance in the Longitudinal Aging Study Amsterdam. Physical performance was measured by a walk test, chair stands and the tandem stand, a measure for balance, and the score varied between 0 and 12. There was a significant strong positive relationship between serum 25(OH)D and physical performance (Wicherts et al., 2005).

6. Non-classical functions of 1,25(OH)2D

Many genes are upregulated by 1,25(OH)2D. These include osteocalcin, osteopontin, calbindin, 24-hydroxylase and many others (Nagpal et al., 2005). On the other side, active vitamin D metabolites downregulate inflammatory markers such as IL-2 and IL-12 and have an antiproliferative effect. They also decrease PTH and PTHrP through a negative vitamin D responsive element (Nagpal et al., 2005). The circulating 1,25(OH)2D is formed in the kidney under stimulation of PTH and negative feedback by serum calcium and 1,25(OH)2D. In extrarenal cells and tissues, 25(OH)D can also be hydroxylated to 1,25(OH)2D under the influence of cytokines. This extrarenal 1,25(OH)2D appears important for the paracrine regulation of cell differentiation and function (Peterlik and Cross, 2005). Vitamin D deficiency plays a role in the pathogenesis of auto-immune disease. Higher sun exposure at an age of 6–15 years was associated with a lower risk of multiple sclerosis. The active metabolite 1,25(OH)2D could prevent auto-immune encephalomyelitis, an animal model of multiple sclerosis. Vitamin D metabolites may also protect against diabetes mellitus type 1

by downregulation of dendritic and Th1 cells, suppression of the antigen-presenting capacity of macrophages and dendritic cells and promotion of Th2 lymphocytes (Mathieu et al., 2005). Vitamin D also influences β-cell function. Serum 25(OH)D was positively related to insulin sensitivity and negatively related to first- and second-phase insulin response (Chiu et al., 2004).

Several ecological studies have shown a relationship between lower sunshine exposure and higher cancer prevalence or cancer mortality, e.g. for colon and breast cancer. Many genes in prostate, colon and breast cancer cells are positively or negatively regulated through the vitamin D receptor (Nagpal et al., 2005). In general, 1,25(OH)2D suppresses proliferation and stimulates differentiation of cancer cells, but some exceptions may exist (Van den Bemd et al., 1995).

7. Conclusion

In conclusion, the active metabolite 1,25(OH)2D has pleiotropic effects on one side through the vitamin D receptor and vitamin D responsive elements of many genes and on the other side rapid non-genomic effects through a membrane receptor and second messengers. The hydroxylation of 25(OH)D to 1,25(OH)2D occurs in the kidney under the influence of parathyroid hormone, or in extrarenal cells and tissues under the influence of cytokines. Active calcium absorption from the gut depends on an adequate formation of 1,25(OH)2D and intact vitamin D receptor. The same is true for osteoblast and osteoclast activity and longitudinal bone growth. Bone mineralization mainly depends on ambient calcium concentration. Prevention of auto-immune diseases such as multiple sclerosis and diabetes type 1 depend on sufficient 1,25(OH)2D at a certain critical age. Locally synthesized 1,25(OH)2D stimulates cell differentiation in a paracrine way and might play a role in cancer prevention.

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