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VITAMIN D, AGING AND CHRONIC DISEASES

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Abstract: Aging is a complex event and several separate molecular mechanisms lead to the same endpoint, normal or premature senescence. The interrelationships between different signaling systems involved in aging remain speculative. Aging is characterized by gradual loss of stress tolerance due to an accumulation of DNA and protein damages. According to a dominating hypothesis the damages are caused by the oxidative stress. The repair mechanisms, anti-oxidative enzymes, IGF signaling, sirtuins and NF□B play an important role in the premature aging. Vitamin D is a prohormone and currently there are at least three cholecalciferol hormones (CHs): 1□-calcitriol (1□,25(OH)2D3), calcidiol (25OHD3) and 24-calcitriol (24,25(OH)2D3). A combination of 1□-calcitriol and calcidiol seems to act synergistically in the target cells regulating several functions including aging. The physiological circulating levels of calcidiol or 1□-calcitriol alone are not sufficient for biological responses. There is a reciprocal relationship between these metabolites (a negative feedback control), which seems to regulate the endocrine balance of CHs. CHs have been demonstrated to be key factors in the regulation of most of these mediators and therefore they play a central role in aging. It seems that an optimal calcidiol serum concentration might delay aging. On the contrary, calcidiol imbalance may lead to a premature aging and earlier appearance of chronic diseases (osteoporosis, cancer, atherosclerosis, neurodegenerative disorders etc) as a sign of aging. Epidemiological studies on the role of hormonal forms of vitamin D3 in chronic diseases are inconsistent. There are several reasons to this inconsistency and one of the reasons might be a non-linear dependency on calcidiol serum concentrations. A U- or J-shaped risk curve is typical to hormones such as steroids, thyroid hormones, retinoids as well as cholecalciferol hormones. The phenomen is known as hormesis. CHs seem to have harmful effects on health and accelerate aging both at low and high serum concentrations. This suggests that there is an optimal serum concentration of calcidiol, which delays aging. The elderly people are in a high risk of vitamin D insufficiency with aging (cholecalciferolpause), because they are not exposed to sun and their skin has a low capacity to produce vitamin D3. Selected studies based on several health outcomes suggest that the optimum is between 40-80 nmol/ L (16-32 ng/ml). This level is reached, if the daily vitamin D dose in the elderly is 10-20 μg. However, more studies are needed on several common degenerative diseases, before the final vitamin D recommendations to elderly can be made.

Key words: Elderly, nutrition, counseling.

Introduction

Aging is a complex biological process at molecular, cellular and organismal level influenced by genetic and environmental factors. It is generally characterized by a declining ability to respond to stress, an increasing homeostatic imbalance and an increased risk of aging-related diseases. Although an accumulation of cellular and DNA damages mainly caused by oxidative stress (wear and tear) might be the cause of aging, the weakening repair mechanisms may also play an important role, especially in carcinogenesis (1).

Around mid-life, even in the absence of chronic diseases vitamin D metabolism begins to change towards an insufficiency (2). Serum concentration of 25OHD3 (calcidiol) decreases, calcium absorption in the intestine diminishes, and calcium bioavailability declines. This in turn stimulates PTH (parathyroid hormone) secretion (3, 4). The mild hyperparathyroidism associated with aging stimulates bone turnover leading to accelerated osteoporosis. In the past decades, our knowledge about vitamin D3 and its biological activities has significantly developed: Its hormonally active forms are not only bone hormones, but they have a wide spectrum of actions in health and diseases (5). During the last decade, there has been accumulating evidence, that an excess production of 1α,25 (OH)2D3 (calcitriol) plays a crucial role in the premature aging caused by FGF-23 (fibroblast growth factor-23)/ klotho mutations (6). On the other hand, a vitamin D3 insufficiency seems also to enhance premature aging (7). The negative vitamin D3 balance with aging in turn accelerates premature aging phenomena as well as the expression of chronic

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diseases(8). Therefore a vitamin D3 supplementation in elderly is important, but it is not an easy task, because the most optimal supplementation is difficult to define. This chapter deals with the complexity of vitamin D3 supplementation with a special reference to the unique endocrinology of cholecalciferol hormones and their role in aging and aging-related diseases.

Functions of cholecalciferol hormones: dual hormone theory

Our understanding of how vitamin D mediates biological responses has entered a new era. Calcium homeostasis and bone are no more the only targets of vitamin D, but practically all cells in the body contain vitamin D receptor (VDR). Therefore vitamin D is involved in a wide range of diseases (7, 9). The main functions of hormonal forms of vitamin D could be classified as follows: 1. Anti-proliferation action. 2. Differentiation (10) 3. Antimicrobial innate immunity (11) 4. Immunomodulation (5) 5. Apoptosis (12) 6. Genomic stability (13) 7. Calcium homeostasis and 8. Detoxification (14-16). All of these functions are important in aging.

Since 1968, there was an general agreement that the active form of vitamin D3 was 25OHD3 (calcidiol) (17), but this dogma was rejected 1971, when 1α,25 (OH)2D3 (calcitriol) was isolated and found almost 1000-fold more active when assayed on the molar basis (18). The latter view was strengthened, once VDR binding assays with radioactive ligands were developed and VDR was cloned (19). As a consequence, the basic dogma in vitamin D endocrinology was that calcidiol is an inactive metabolite hydroxylated by CYP27A1 (25-hydroxylase) mainly in the liver. Calcidiol is thereafter activated in the kidney via 1α-hydroxylation (CYP27B1) to the biologically active 1α,25 (OH)2D3 (1α-calcitriol). All forms are carried in the blood bound to vitamin D binding protein (DBP), calcidiol having the highest affinity and the highest proportion as protein bound. The prevailing hypothesis was that the free 1α-calcitriol was the biologically active hormone. Today, almost every detail of the classical dogma has changed, or there are proposals for alternative explanations. 25-hydroxylation does not occur only in the liver, but in many organs including skin, which may contribute significantly to the serum calcidiol concentration (20). Even though kidney seems to be the major source of serum 1α-calcitriol, the extra-renal calcitriol production seems to be physiologically important as an auto- and paracrine factor (21-25). These findings led to the conclusion that the local production of 1α-calcitriol regulates cell growth and differentiation. However, it is unlikely that 1α-calcitriol could be responsible for the effects, because its concentration is too low intracellularly and in the serum (26). The half-life of calcidiol in the circulation is about two weeks, while that of α-calcitriol is less than four hours (27). In vitro, 1α-calcitriol can produce biological responses in serum-free medium being in favor of the "free" hormone hypothesis (28). However, the concentrations of 1α-calcitriol needed for the responses are usually 100-1000-fold over the physiological ones. Moreover, the serum level of calcidiol is approximately 1000 times higher than that of 1α-calcitriol. Only 0.04% of calcidiol and 0.4% of 1α-calcitriol are free in plasma and the rest are tightly bound to either a vitamin D binding protein or serum albumin (29). This means that the concentration of the free calcidiol is 100 times higher than that of the free 1α-calcitriol. Based on the "free hormone" hypothesis, calcidiol is accessible to the target cells is 100 times more than 1α-calcitriol, but the absolute concentrations of free hormone in serum are far below the concentrations known to give any biological response. On the other hand, based on the "bound hormone" hypothesis (30-32), the bound calcidiol that can be taken up by the target cells has approximately 1000 times higher serum concentration than that of 1α-calcitriol. If calcidiol is inactive metabolite, it would competitively inhibit the action of 1α-calcitriol. The binding affinities of the metabolites to the VDR have been measured in different cell types with varying results, but the binding affinity of 1α-calcitriol is only approximately 50-fold higher than that of calcidiol (33) suggesting that in the physiological situation most of the VDR molecules are occupied with calcidiol.

In order to solve the calcidiol enigma, we planned a set of experiments with calcidiol, where the role of calcitriol was eliminated. Once calcidiol and 1α-calcitriol enter the target cell, they bind to the VDR (34). Calcidiol may also bind to 1α-hydroxylase and be converted to 1α-calcitriol. We eliminated 1α-hydroxylase pharmacologically and genetically. By blocking 1α-hydroxylase activity with a specific enzyme inhibitor, we have demonstrated that calcidiol itself can regulate gene expression in human primary prostate stromal cells (35) and mouse primary prostate cells (36). In addition, we demonstrated that calcidiol can promote the activity of 24-hydroxylase gene promoter in MCF-7 human breast cancer cells and inhibit the growth of LNCaP human prostate cancer cells (35). We also isolated primary cells from the kidney and skin of 1α-hydroxylase knockout mice. In the 1α-hydroxylase knockout kidney and skin cells, we verified the gene regulatory action of calcidiol (37). In addition, calcidiol seems to inhibit precancerous and alveolar lesions in mouse mammary organ culture systems derived from 1α-hydroxylase knockout mice (38). All the data mentioned above demonstrate clearly that calcidiol has an inherent hormonal activity regulating genes and cell proliferation. Our findings are supported by studies of Peng et al (38) showing that calcidiol (100 nM) inhibited growth of mammary cancer.

All the products of 24-hydroxylation (CYP24) were earlier presumed to be inactive degradation products. However, there is accumulating evidence suggesting that

at least 24,25(OH)2D3 (24,25-cholecalciferol or 24-calcitriol) might be biologically active in chondrocytes (39). This action seems to be mediated by membrane receptor (mVDR), because the effect can be detected also in nuclear VDR-/- cells. 24-calcitriol seems to act also through the nuclear VDR stimulating differentiation of human osteoblasts as well as bone mineralization (40). Furthermore, 24-calcitriol seems to be necessary for bone fracture healing, since the callus formation is impaired in CYP24-/- mice (41, 42). It can be concluded that there are at least 3 hormonally active forms of vitamin D3: calcidiol, 1α-calcitriol and 24-calcitriol (Fig 1). We proposed recently that these hormones should be called cholecalciferol hormones (CHs) (43). There is a good argument for each term: The prefix "calci-" is already used in the names of individual hormones (calcidiol etc). On the other hand, the basic structure is known as cholecalciferol and it favors the latter name. It is important to avoid the use of the term "vitamin D", since there is no biologically active vitamin D, but only CHs are biologically functional.

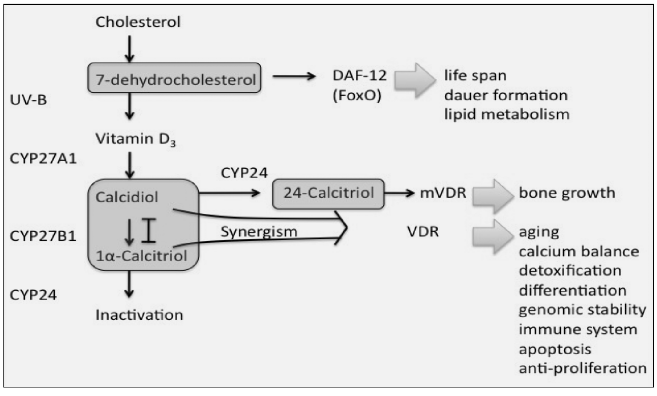
It is possible that new active cholecalciferol hormones will be found in future (44). Hydroxylations occur at all positions from 20 to 28 except for positions 21 and 27. Therefore, there are several hormone candidates to be tested for their activity in physiological situations.1α,20 (OH)2D3 metabolized by CYP11A1 is hormonally active metabolite in skin keratinocytes (45) and its activity is VDR-dependent.

A dilemma in cholecalciferol hormone endocrinology has been the reciprocal feedback between serum calcidiol and 1α-calcitriol. Chronic administration of 1α-calcitriol increases the metabolic clearance of calcidiol (46, 47) and decreases its production (48) with consequent depletion of calcidiol stores. On the other hand, administration of elevated doses of vitamin D3 or calcidiol increases the metabolic rate of 1α-calcitriol (49). The "hormonal calcidiol" concept helps to understand the feedback system, since both hormones seem to be active and able to regulate the mutual expression. The physiological significance of the negative feedback (Fig.1) could be that the hormonal sum effect of serum/intracellular calcidiol and 1α-calcitriol is under control. As a consequence, 24-hydroxylated products in the serum increase always, when either calcidiol or 1α-calcitriol increases in the serum. Therefore, an increase of serum 24-calcitriol might be the best indicator of an overdose of oral vitamin D3.

A strong argument against "hormonal calcidiol" concept is the phenotype of the 1α-hydroxylase knock out mice (CYP27B1-/-), which is quite similar although not identical to that of VDR-/- mice (7). The CYP27B1-/- mice have an elevated serum calcidiol concentration, but it cannot prevent the phenotype. This discrepancy can be explained with our recent finding on the mutual synergistic action of calcidiol and 1α-calcitriol (36). In CYP27B1-/- cells, calcidiol and 1α-calcitriol show a synergistic action on the CYP24 expression. We treated 1α-hydroxylase knockout kidney and skin cells with both calcidiol and 1α-calcitriol at different concentrations and found that the combined effects of both metabolites are significantly higher than the sum of the effects of either alone. We do not know the mechanism of the synergism, but a possible explanation could be homodimerization of VDR occupied with the two ligands (calcitriol and calcidiol). Calcidiol could bind to AP-site and 1α-calcitriol to GP-site causing different conformational changes allowing apoVDR homodimer stabilization (50-56). It is apparent that the coactivator complex associated with homodimer is different as that bound to VDR-RXR heterodimer suggesting that the genes regulated are also different. The hormonal synergism suggests that both calcidiol and calcitriol, in fact, are important hormones and they act in concert. Therefore, calcidiol with its variable serum concentrations has always been clinically and epidemiologically more important than 1α-calcitriol with rather stable serum concentrations. Within the cell, neither the concentration of calcitriol nor calcidiol alone is sufficient for a significant physiological response, but in combination they can cause a perfect response (37). This we call "dual hormone theory". The dual cholecalciferol hormone synergism is schematically presented in Fig 1.

Figure 1

A simplified endocrine system of cholecalciferol hormones. There are three active hormonal forms calcidiol (25(OH)D3, 25-hydroxy-cholecalciferol), 1α-calcidiol (1,25(OH)2D3, 1α,25-dihydroxy-cholecalciferol) and 24-calcitriol (24,25(OH)2D3, 24,25-dihydroxy-cholecalciferol) . Calcidiol and 1α-calcidiol act synergistically via vitamin D receptor (VDR) and they have a negative mutual feeback regulation (|—|). 24-calcitriol acts probably via membrane vitamin D receptor (mVDR). DAF-12 (dauer formation protein-12) regulates life span of C. elegans and it is a homolog of mammalian VDR and FoxO (forkhead box protein). The putative ligand of DAF-12 is 7-dehydrocholesterol. CYP27A1= 25-hydroxylase, CYP27B1 = 1α-hydroxylase, CYP24 = 24-hydroxylase. For the details and references see text



An unsolved dilemma in the VDR action is the regulation of hair cycle. Alopecia is a part of the phenotype of many patients with hereditary vitamin D-resistant rickets (HVDRR) caused by VDR mutation (57). VDR-KO mice develop their first coat of hair normally, but begin to loose their hair rostrally at the age of 6 months and hair loss is complete at the age of 8 months (43). The reinitiation of anagen following the first cycle or after the depilation is impaired (58). Reconstitution of the VDR in keratinocytes to the VDR-KO mice reverses the defect in hair cycle and the mice do not develop alopecia (59). Correction of metabolic disturbances with a high calcium diet prevents rickets and hyperparathyroidism but does not prevent the alopecia (60), which suggests that alopecia is not calcium-dependent. Because CYP27B1-/- (1α-hydroxylase deficient) mice show rickets, growth retardation, osteomalacia, hypocalcemia, hypophosphatemia and hyperparathyroidism, but no alopecia (61), it seemed that the development of alopecia is VDR-dependent, but not dependent on calcitriol. This raised a question whether hair cycle is regulated by apoVDR or there is an unknown ligand of VDR for hair follicle development? Because serum calcidiol concentration increases in CYP27B1-/- mice, it is possible that this high calcidiol concentration would be sufficient to maintain the hair cycle.

U-shaped serum calcidiol level-disease response

A new aspect in cholecalciferol hormone endocrinology is the U-shaped dose response to serum calcidiol concentration of some diseases and aging phenomena. This is also known as hormesis (62): both insufficient and excess concentrations of CHs are harmful to health. Hormesis is the term for usually favourable biological responses to low exposures to toxins, toxic substances and other stressors. The concept has been explored extensively in respect of aging (63, 64). According to the hormesis paradigm, agents induce dose-response relationships having two or three distinct phases (biphasic, U-, J- or reversed U-shaped= bell-shaped). A mild stress exposure such as sun radiation usually has anti-aging effects (65). A U-shaped hormesis is typical to all natural ligands of nuclear receptors such as retinoic acid, thyroid hormones and steroids. A hormetic response to serum calcidiol concentration is reported in some epidemiological studies: risk of prostate cancer (66, 67), all cause deaths and cardiovascular deaths (68-71). Recently, more and more U-shaped dose responses between serum calcidiol and disease have been found such as small-for-gestational-age births (72), falls and fractures in elderly women (73), schizophrenia (74), mammary cancer (75), common single nucleotide polymorphism (76) and deafness (77, 78). Also the aging process in general shows an U-shaped response to cholecalciferol hormones (7): both low and high action of CHs enhance aging phenomena such as skin aging, osteoporosis, ectopic calcification, immunodeficiency, muscle atrophy and loss of hearing. Another type of vitamin D hormesis is a reversed response. Usually higher serum calcidiol concentrations seem to protect against cancer as mentioned above, but sometimes they increase the risk of cancer such as oesophageal cancer in men (79), pancreatic cancer (80). In skin, low doses of 1α-calcitriol increase epidermal cell proliferation, whereas high doses inhibit it (81). Thus, it seems that there is an optimal serum concentration of calcidiol, which may delay aging and prevent aging-related diseases. Based on the epidemiological studies mentioned above, the optimal concentration is approximately 40-80 nmol/L, but it may be different in different diseases and therefore more studies are needed. The U-shaped response has been contested by Grant (82). He argues that the diseases showing U-shaped response relation with serum calcidiol are not representative cases, because there are more studies showing reversed linear relationship and all meta-analyses show a monotonic decrease of hazard ratio with increasing serum calcidiol. The key problem is, that linear regression analyses tend to give linear dependency or no relationship and therefore U-shaped responses may not be found, however IARC working group found U-shaped responses in their meta-analyses (69). In most studies, the material is too small to find statistically significant U-shaped response a good example being the study of Faubel-Badger (67), which shows similar U-shaped association (but statistically not significant) as our study (66). Behind this controversy might be the basic epidemiological analysis dividing comparable groups in quartiles or quintiles. The differences of serum concentrations between the groups are so small, that they are physiologically insignificant. Defined serum calcidiol concentrations (e.g. 20 nmol intervals) (66) might be more relevant. The U-shaped dose response is typical to all hormones and it seems that CHs are similar. An apparent explanation for U-shaped response to CHs is vitamin D resistance at higher concentrations (83).

Age-related degenerative diseases

Epidemiological and clinical studies show that the serum 25-hydroxyvitamin D (calcidiol) levels are inversely associated with osteoporosis (84, 85), muscle weakness (86), common solid cancers (8), including breast cancer (87, 88), prostate cancer (89), ovarian cancer (90), and colorectal cancer (91, 92) and many other chronic diseases, such as cardiovascular diseases (71), hypertension (93, 94), psoriasis (95), rheumatoid arthritis (96), diabetes (97-99), respiratory infections (100), multiple sclerosis (101, 102) and several

neurodegenerative diseases (43). The potential of cholecalciferol hormones in the prevention and treatment of degenerative diseases is widely reviewed by Armin Zittermann (103, 104). In some degenerative diseases, CHs are directly involved in the basic mechanism of the disease such as negative regulation of renin-angiotensin system in hypertension (105, 106), insulin regulation in diabetes (98, 107, 108), several osteotrophic genes (osteoporosis) (109) anti-microbial peptides (infections) (11) and mitotic control in cancer and psoriasis (10). However, in most of the diseases the mechanism of action of CHs is unknown. It is possible that these diseases reflect the cholecalciferol hormone-dependent aging process as described below. Thus, an increased risk of chronic diseases means that the degenerative disease genetically disposed appears earlier because of premature aging in the presence of cholecalciferol hormone imbalance. In conclusion, several chronic degenerative diseases are at least partially vitamin D-dependent and their early appearance is a sign of premature aging as well as often a sign of vitamin D imbalance.

Cholecalciferolpause and supplementation

The insufficiency of CHs during aging could be called cholecalciferolpause like andropause and menopause. The main cause for the common vitamin D insufficiency in elderly (110-112) seems to be a decreased skin production of vitamin D3 due to lack of 7-dehydrocholesterol (113, 114). The dimished skin production in elderly is often aggravated by changes in lifestyle. Many older people are homebound, and when exposed to the sun often wear protective clothing. The cholecalciferol hormone balance develops more and more negative with aging, and this hormone insufficiency in turn may facilitate aging processes.

Intestinal absorption of vitamin D3 is not significantly affected in elderly people in the absence of diseases associated with intestinal malabsorption (115). Synthesis of calcidiol does not appear to be influenced by aging (2). The effect of aging on serum concentration of 1α-calcitriol is controversial: A number of studies suggest either decrease, no change or increase (see review (2)). However, bone responsiveness to CHs seems to decrease with aging (116). This is most likely due to a decrease of VDR concentration in target tissues. Also intestinal calcium absorption is affected in the elderly (117). As a consequence of the hypovitaminosis D, a mild hyperparathyreoidism is often seen in elderly, which in turn stimulates bone turnover accelerating osteoporosis.

The main reason for vitamin D supplementation in elderly is to reduce bone fracture incidence (118). There are 3 factors playing role in the risk of bone fracture: 1) muscle strength (119), 2) balance and 3) bone mineral density (118). Several studies suggest that cholecalciferol hormones regulate muscle strength (119-121). This is apparently due to the regulation of calcium balance within the muscle cell. It has been proposed that vitamin D-deficient elderly people are at a higher risk of falling because of impaired muscle tonus. Much less attention has been paid to the role of balance in the risk of falling during aging. The balance deficit is one of the first signs of aging. Using aging VDR-KO mice, we found the their balance deficit developed faster than in normal mice (122). Because otolites in semicircular canals contain calcium, it is possible that their development and turnover needs CHs and VDR, which we demonstrated in the epithelium of crista ampullaris. It is interesting that the sensorineural hearing loss is also associated with hypoparathyroidism (123, 124). The vitamin D supplementation may not effectively increase the bone density, but it is extremely important in preventing the further development of osteoporosis in elderly. Since the mild PTH elevation in elderly is the main factor for osteoporosis risk, the supplementation needed should be effective to suppress PTH. For fracture risk reduction in the elderly, the weight of evidence indicates that the minimum amount of serum calcidiol needed is 75 nmol/L or more (118).

In the elderly, the prevention of chronic diseases with vitamin D supplementation (see below) may not be as important as earlier in life, because even with the most optimal supplementation only few healthy months (not years) can be achieved. Certainly, bone fracture may be an important invalidity limiting the life span and the quality of life, but the most interesting new areas of preventive medicine in the elderly are the mood, senses and neurological degeneration (Table 1).

Table 1
Indications for vitamin D supplementation in the elderly

Indications with substantial evidence
1. Prevention of osteoporosis (236)
2. Prevention of hip fractures (237)
3. Muscle strength (120)
4. Home- or hospital-bound elderly (238)
Promising indications
5. Vitamin D-dependent hearing loss (77, 128, 129)
6. Age-related macular degeneration (AMD) (125)
7. Seasonal affective disorder (SAD) (136, 139)
8. Depression of the elderly (137, 138)
9. Alzheimer's disease (133)
10. Parkinson's disease (161)
11. Cognitive ability (132, 134, 135)

The degeneration of senses with aging might be faster in vitamin D insufficiency than in normovitaminosis. A common reason for blindness in the elderly is age-related macular degeneration (AMD). There is preliminary evidence suggesting that cholecalciferol hormones may protect against aging associated macular degeneration (AMD) (125). Vitamin D-dependent deafness has been known for almost three decades and its reversal with

vitamin D supplementation (77, 126-128). In the VDR-KO mice, we found unilateral loss of hearing as a sign of premature aging (129). These animals also showed signs of mild balance deficits (122), but not any loss of gustatory nor olfactory senses (130). A degeneration of senses is clearly associated with aging. The apparent cause of deafness might be disturbances in calcium metabolism of the inner ear, and therefore it is also associated with hypoparathyroidism (123, 124). It is interesting that also hypervitaminosis D is associated with loss of hearing (78). In conclusion, the senses, some of which are calcium-dependent, are more likely affected in the cholecalciferol hormone disturbances.

As to the quality of life of the elderly, putative neuroprotective properties of vitamin D are of interest in the future (43). 1α-Calcitriol seems to enhance the gluthatione content of neurons and to protect them from the reactive oxygen species (131). A vitamin D insufficiency seems to be associated with the cognitive impairment in the elderly and Alzheimer patients (132-135). One of the most important reason for poor quality of life in the elderly is depression, therefore relevant studies on the role of vitamin D are needed. Preliminary studies suggest that sufficient administration of vitamin D would improve seasonal affective disorder (SAD) as well as other types of depression (136-139). Vitamin D sufficiency may delay the appearance of neurodegenerative diseases (43, 140, 141) (Table 1).

Vitamin D3 photosynthesis during the sun exposure is enormous (142). It is obvious that the high photosynthesis rate became a problem to living organisms. Part of the problem is solved by photodegradation of the precursors of vitamin D3, but all living organisms have also developed an effective detoxification system based on CYP24, 24-hydroxylase, which initiates a series of hydroxylations leading finally to the excretion in the kidney as calcitroic acid (143). There is a basic difference in the physiology of vitamin D transport in the skin vs intestine. Absorption of the oral vitamin D in ileum is without any control up to 100%. The intestinal absorption is fast and the 25-hydroxylation in the liver is not rate limited, if the body stores are within the normal range (144). This in turn means that the serum calcidiol concentration shows a peak value shortly after oral administration of vitamin D. This kind of daily variation is smaller, when vitamin D is produced in the skin exposed to sun. The sun exposure slowly increases serum calcidiol reaching maximum one or two weeks later (145). This suggests that the negative feedback systems of CHs (discussed above) are probably less activated, if the vitamin D is obtained from the skin. Therefore it seems that sun exposure or narrow band UV-B irradiation could be the method of choice for elderly people. However, the significant reduction (up to 70%) of vitamin D production in the aging skin may limit the efficiency of UV-irradiation.

The oral vitamin D supplementation in the elderly is compromised because of the increased incidence of intestinal malabsorption diseases with aging. However, a similar response in serum calcidiol (22 nmol/L) was found both in young and old men to daily dose of 20 μg of vitamin D3 (115). Very similar result was found in a study on Finnish elderly women (65 to 85-year-old) (146). A daily dose of 5, 10 or 20 μg vitamin D3 increases serum calcidiol 11, 14 and 24 nmol/L within 6 weeks, respectively. The more vitamin D is given, the smaller is the relative net increase in serum calcidiol. This is apparently due to the negative feedback system caused by the daily peak values of calcidiol, since it activates 24-hydroxylase (83). Furthermore, vitamin D deficient people respond more efficiently to supplementation than healthy subjects with an adequate vitamin D status (147). This suggests that at the population level the general recommendations for supplementation should be cautious. The upper limit for the general recommendation for the elderly could be higher than for the younger ones, but not higher than 20 μg (800 IU). Higher recommendations are today common (148, 149), but more clinical studies are needed, before these recommendations are practised. A good indicator of the too high dosage would be serum 24OH metabolites, because the induction of 24-hydroxylation can be regarded as a detoxification defence system as discussed above. A solution for vitamin D administration for the elderly (as well as for younger people) would be a vitamin D band-aid or topical patches similar to HRT, which theoretically should give an even calcidiol serum concentration (plateau) without peak values. This idea would be worth to study more in detail.

Role of cholecalciferol hormones in aging

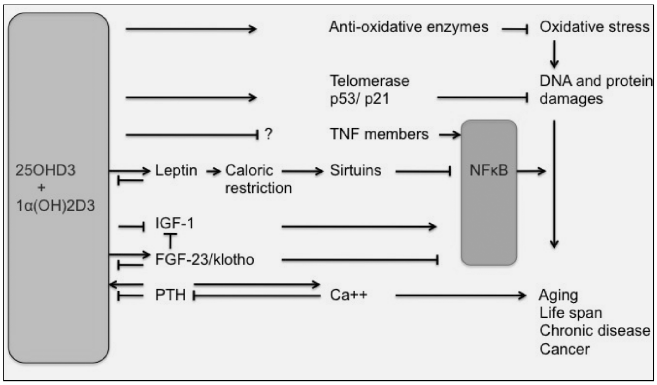
The main models for premature aging come from inherited progeroid syndromes. Segmental progerias, such as dyskeratosis congenital, Werner´s disease, Bloom syndrome and ataxia teleangiectasia display symptoms of accelerated aging, mainly due to reduced DNA repair and increased genetic instability (150). Some progerias display symptoms—such as alopecia, osteporosis and fingernail atrophy—associated with shortened telomeres. It is interesting that the latter symptoms are also typical to vitamin D3 deficiency.

Within the last decade, 1α-calcitriol became a key mediator of aging. Klotho and fibroblast growth factor-23 (FGF-23) mutations in human and experimental animals caused a premature aging (151-153). The over-expression of Klotho can extend the lifespan of mice (154). Klotho protein can repress insulin/IGF-1 signaling and induce insulin resistance. Recently, Maekawa et al (155) demonstrated that the Klotho protein could inhibit the TNFα-induced activation of NFκB signaling and

subsequently reduce the inflammatory reaction. It appeared that the aging process caused by klotho or FGF-23 mutations was 1α-calcitriol-dependent (6). Although dysregulation of cholecalciferol hormones, especially the up-regulation of 1α-hydroxylation, can explain all the aging phenomena caused by FGF-23 mutations, the role of calcium signaling cannot be totally excluded (156), because almost all the aging phenotype changes could be corrected by reducing serum calcium as well as by deletion of 1α-hydroxylase gene. A dysregulation of calcium signaling seems to play important role in the development of aging-related diseases (157) and neurodegeneration (158). It is important to note that, FGF-23/klotho seems to be the main negative regulator of 1α-hydroxylation. In conclusion, elevated 1α-calcitriol and/or calcium seem to mediate all aging phenomena associated with the impaired FGF-23/klotho signaling (Fig. 2).

Figure 2

A model of the aging mechanisms regulated by cholecalcipherol hormones, calcidiol and 1α-calcidiol. → = stimulation. —| = inhibition. ? = regulation of the different members of TNF family is variable. For the details and references see text



Aging can be understood as a process of the homeostatic imbalance. Homeostasis means the ability or tendency of an organism or cell to maintain internal equilibrium by adjusting its physiological processes. An imbalance will lead to aging, diseases and death. According to hormonal theories of aging, the gradual or rapid decline hormones (hormonal imbalance) such as androgens and estrogens may initiate aging (159). Like estrogen decline (menopause) or androgen decline (andropause) also CHs are known to decrease significantly with aging (cholecalciferolpause) (2, 7). Another possibility to homeostatic disturbance is the immunological imbalance. CHs are known to regulate acquired immune systems at several levels (109). 1α-Calcitriol is known to suppress as well as stimulate immune responses (160). The consequence of imbalance of defense mechanisms is immunosenescence. Also neurohumoral imbalance leads to aging of nervous system. It is possible that CHs exert a combination of neurohumoral and immunological action in the central nervous system (161). CHs are often classified as neurosteroids (162), a more appropriate term could be neuroactive secosteroid (43). In the studies of aging, the hormesis concept has often been applied. A mild stress to cells and organisms induce adaptive stress resistance, which is called hormesis. The hormesis includes that the effect of low dose may be beneficial, whereas a higher dose is pleiotropically harmful. A mild irradiation can delay aging and, in fact, have some health promoting effects in contrast to heavy irradiation (65). The U-shaped hormesis to varying serum concentrations of calcidiol is discussed above. Hormesis may explain, why both very low and high serum CHs enhance aging.

The anti-aging effect of low caloric diet has been known for about century. Low caloric diet and fasting delays aging and the effect is thought to be mediated by sirtuins (163). Sirtuins are homologs of yeast Sir2 (silent information regulator two protein) (164). They are histone deacetylases or ADP-ribosyl transferases and locate in nucleus, cytoplasm or mitochondria. The mammalian seven Sirtuins have important functions in the regulation of metabolism, growth, differentiation, cell survival, aging and life span (165). The anti-aging effect of caloric restriction seems to be mediated especially by Sir1 (166, 167). Sirtuins are known to enhance autophagy (168) and suppress NFκB activity (169), which are strongly involved in aging processes (see below "disposable soma"). So far, there is no evidence that CHs could directly regulate sirtuins, but there is an indirect hormonal connection. Serum calcidiol correlates inversely with serum leptin (170) and may regulate leptin secretion. Lean phenotype is typical to VDR-KO mice (171). Because leptin controls appetite, it will lead to caloric restriction and in turn to an induction of sirtuins. Leptin is a negative regulator 1α-hydroxylase (172) suggesting that there is a feedback loop between 1α-calcitriol and leptin. Leptin is involved in brain aging, Alzheimer's disease (173). There is another interesting hormonal connection: Leptin seems to regulate also PTH (170). On the other hand, PTH seems to be involved in the regulation of aging (174-176) and serum calcium might be the key mediator of the aging. Both primary and secondary hyperparathyroidism increase death rate independently of vitamin D status or general health factors (177). A model of the role of these hormonal factors on aging is schematically shown in Fig 2.

Vitamin D has played a significant role in evolution (178). During human evolution, selection has been effective e.g. for the skin color, because vitamin D deficiency will lead to pelvic deformation and difficulties in childbirth. The light skin type is able to respond to UV-B up to 50-fold more effective. Aging of organisms is a precondition for evolution, because without death no evolution is possible. There are three mainstream

evolutionary hypotheses of aging: 1) The accumulation of deleterious somatic mutations and reduced ability to repair DNA (179). The actions of CHs fit well to this hypotesis. 2) Antagonistic pleiotrophy referring to genes that enhance reproductive success early in life, the by-product of which is later enhanced aging and death (180). A similar hypothesis was later defined as an evolutionary compromise by Darwinian medicine (181-186): In species with long life-span, the repair mechanisms need to be less active than in short-living species, because the same genes are involved in aging-related diseases such as cancer, and therefore too active repair mechanisms would cause cancer later in life. The pleitrophic shift of the action of the GH/insulin/IGF-1 signaling system during aging is a good example of the antagonistic pleiotrophy hypothesis. CHs are able to regulate IGF signaling as shown below. 3) A disposable soma says that finite food energy is preferentially used for reproduction, but compromises repair (187).

Accumulation of deleterious mutations

Harman (188) in 1956 formulated his free-radical theory of aging and later identified mitochondria as the major endogenous source of oxidative stress (189). Aging mechanisms include oxidative stress, DNA mutations and shorter telomeres (190). Antioxidant defense, DNA repair and telomerase temper the effects of these deleterious events. Active forms of vitamin D seem to regulate some antioxidant mechanisms (13, 191-194). Calcidiol increases the activity of superoxide dismutase, gluthatione peroxidase as well as that of catalase (Fig 2). Thus, cholecalciferol hormones may to certain extent directly oppose the oxidative stress, decrease the risk of DNA mutations and increase the genomic stability (13).

The length of telomeres is dependent on the activity of telomerase reverse transcriptase (TERT). Along with cell divisions and aging telomeres gradually shorten due to insufficient reverse transcription. A combination treatment with 1α,25 (OH)2D3 and 9-cis-retinoic acid inhibits human telomerase reverse transcriptase (195). It was proposed that this could be a mechanism to inhibit the cell proliferation and to be a tool against carcinogenesis as well as it may therefore extend the life span. Cancer cell, in order to become immortal, acquire the capacity of expressing telomerase (196, 197). Short telomeres lead to senescence (198) and an efficient telomerase activity is necessary for longevity. Therefore the inhibitory effect of 1α-calcitriol on TERT raises the question, whether it inhibits or accelerates aging?

The tumor suppressor protein, p53, is essential for the detection of DNA damages and their repair, its inactivation enhances cancer development, whereas its overexpression leads to premature aging (199, 200). The phosphorylation of retinoblastoma protein (Rb) does not occur in the cells with DNA damages and they cannot enter the cell cycle, where p21 regulated by p53 plays a central role. CHs seem to up-regulate the expression of both p21 and p53 (201) and enhance apoptosis. Accordingly, we found the expression of p53 was significantly decreased in the aged VDR-KO mice (202). It is interesting that VDR is induced by DNA damage as well as by p53, which may mediate the effect of DNA damage on VDR expression (203). The lower level of p53 expression in VDR-KO mice might explain the higher susceptibility of their skin to UV-induced cancer (204). Collectively, these data suggest that p53 and p21 might be key mediators of anti-aging effects of CHs (Fig 2).

It has been demonstrated that insulin like growth factor-1 (IGF-1) deficiency increases life span (205, 206). CHs seem to regulate the expression of IGF-1 and IGF binding proteins (IGFBPs) (207, 208). The basal and stimulated IGF-I and II production is inhibited by 1α-calcitriol (209). We found that IGF1 receptor (IGF1R) was down-regulated in VDR-KO mice suggesting a lower IGF activity (202), but the aging of these animals was enhanced. The activity of growth hormone (GH)/insulin/IGF signaling is at least partially mediated by catalase and superoxide dismutase and therefore the oxidative stress is antagonized (1). Insulin/IGF-1 signaling can also enhance NFκB signaling (see below) (206) and subsequently potentiate the aging process and aggravate age-related degenerative diseases. However, the final output of cholecalciferol hormones on IGF-1 signaling system remains unknown, because there are opposite regulations.

Disposable soma

The disposable soma theory on aging states that each individual has to allocate limited energy resources to either maintenance of soma or reproduction (187). This trade-off between reproduction and longevity was shown to exist also in humans (210). The original theory was genetic. A compatible non-genetic theory called entropic host defense catastrophe has been recently proposed (211).

In Caenorhabditis elegans, DAF-12 appears to be a decisive checkpoint for many life history traits including longevity (212). DAF-12 is a member of nuclear hormone receptor family and a close homolog of LXR (liver X receptor) PXR (pregnane X receptor), VDR, CAR (constitutive androsterone receptor) and FXR (farnesoid X receptor) all belonging to the subfamily 1. DAF-12 regulates lipid metabolism, stimulates dauer formation and therefore increases life span of C.elegans. It is interesting that 7-dehydrocholesterol is a putative ligand of the DAF-12 (213) (Fig 1). DAF-12 is downstream of signaling systems that translate environmental cues, as well germline signals (214, 215). This positions DAF-12 at the crossing point of maintenace of soma and germline signaling. Also DAF-16 influences the rate of aging of C.

elegans in response to insulin/IGF-1 signaling (216). Both DAF-12 and DAF-16 belong to the mammalian FoxO-family of transcription factors (forkhead box proteins), which comprise more than 100 members (Fig 1). FoxO members have a critical role in the regulation of the immune system, immunosenescence and general aging as anti-aging factors (217). FoxO factors (similarly as VDR) can induce the expression of several anti-oxidative enzymes such as catalase and SOD2 as well as stress resistance inducers (218). FoxO factors play a key role in the maintenance of the energy metabolism, especially glucose balance (219). The activity of FoxO factors is upregulated by a deficiency of IGF-1 signaling (218, 220) suggesting a negative regulation by IGF-1.

Similarly as DAF-12, VDR mediates environmental stimulus (sun irradiation) to the soma and regulates reproduction. VDR and vitamin D metabolizing enzymes expressed inhuman testis (24). Studies on VDR-KO mice demonstrate that a complete lack of cholecalciferol hormone action impairs fertility. Female mice show uterine hypoplasia and an impaired ovarian folliculogenesis (221). The fertility of vitamin D deficient female rats decreases by 75 % (222). This infertility cannot be reversed by calcium repletion suggesting that the effect directly mediated by VDR (223). Aromatase is one of the targets of VDR (224). Also male fertility is reduced, the sperm number is decreased and testicular morphology is changed (224). As described above, CHs are important for cell cycle control, differentiation, calcium balance, detoxification early in the life, thus they control the homeostatic balance. However, excess as well as a lack of CHs seem to increase risk of some degenerative diseases and aging (see hormesis). It seems that VDR acts at the crossing point of reproduction and soma.

NFκB is known to be strongly associated with aging (225), cancer (226) and skin aging (227). The mammalian Rel/ NFκB family includes three Rel proteins and two NFκB (nuclear factor kappa B) components, p50 and p52. These components form dimeric complexes with each other, which are trapped in the cytoplasm bound to several inhibitory proteins (IκBα, IκBβ, IκBε, IκBζ and Bcl-3). Several protein kinases can phosphorylate IκB proteins. Subsequently, the NFκB comples is translocated into the nucleus, where it can transactivate several genes, especially inflammatory and immunomodulatory genes (228). Several pro-aging factors (oxidative stress, innate immunity, TNF family, and Insulin/IGF-1 signaling) can activate NFκB and several anti-aging factors (SIRT1, SIRT6, FoxOs, p53, Klotho and hormetic phytochemicals) can inhibit NFκB activation (211). Members of TNF (tumor necrosis factor) family, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) can regulate adaptive immunity and are up-regulated with aging (229). The effects of CHs are somewhat complex: IL-6 is either downregulated or not regulated (230, 231), whereas TNF-α seems to be up-regulated by 1α-calcitriol (231). Another member of TNF family, RANK (receptor activator of NFκB) has a key role in osteoporosis. The ligand of RANK (RANKL) is regulated by CHs (232-234).

There is no substantial evidence, whether CHs can directly regulate the activity of NFκB. A study using mouse embryonic fibroblasts derived from VDR+/- and VDR-/- mice suggests that VDR may act directly on IκBα expression and thus prevent NFκB activation (235). We found a decreased expression of NFkB in aging VDR-/- mice (202). It is evident that CHs are involved in the regulation of NFκB activity, but the regulation is complex and occurs at different upstream levels. With aging, the imbalance between the negative and positive regulation of NFκB activity may lead to the NFκB-driven entropic senescence and decreased autophagy as proposed by Salminen and Kaarniranta (211). The central role of NFκB and CHs in aging is depicted in Fig 2.

Summary and clinical relevance

There are at least three hormonal forms, cholecalciferol hormones, derived from prohormone, vitamin D. Two hormones, 1α-calcitriol and calcidiol seem to interact synergistically. There seems to be an optimal serum calcidiol concentration (40-80 nmol/L) for disease prevention. Several aging related degenerative diseases (osteoporosis, atherosclerosis, neurodegenerative disorders and cancer) could be effectively prevented with optimal serum calcidiol concentration. If the calcidiol serum concentration is too low, it cannot act hormonally with 1α-calcitriol or if it is too high, it will lead to resistance. An optimal supplementation of vitamin D is crucial for its health effects. Sun exposure or topical patches might be more physiological than oral administration. It seems that elderly people need somewhat higher dose (appr. 20 μg/D) than the younger ones.

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