Review Article

Ageing and vitamin D deficiency: effects on calcium homeostasis and considerations for vitamin D supplementation

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Vitamin D is a fat-soluble, seco-steroid hormone. In man, the vitamin D receptor is expressed in almost all tissues, enabling effects in multiple systems of the human body. These effects can be endocrine, paracrine and autocrine. The present review summarises the effects of ageing on the vitamin D endocrine system and on Ca homeostasis. Furthermore, consequences for vitamin D supplementation are discussed.

Vitamin D is best known for its role in Ca homeostasis. Ageing affects both vitamin D metabolism and Ca homeostasis, with important consequences. In the present review, we outline new insights into the effects of ageing on both the vitamin D endocrine system and Ca homeostasis, which are relevant for clinicians who treat older people. Furthermore, considerations for vitamin D supplementation will be discussed.

Vitamin D metabolism

Vitamin D is a fat-soluble, seco-steroid hormone. The term vitamin D refers to two precursors, i.e. cholecalciferol and ergocalciferol. Cholecalciferol is mostly formed in the skin after exposure to sunlight. In the skin, the precursor 7-dehydrocholesterol is transformed into cholecalciferol under the influence of short-wave UV light[1]. Another source of vitamin D is the diet. Ergocalciferol is generated in yeast and plants and cholecalciferol is produced in fish and mammals. In general, oral vitamin D intake, especially in Europe, is low and depends mostly on cutaneous production of vitamin D for our reserves[2]. The inert precursors are transported to the liver, where they are converted to 25-hydroxyvitamin D_3 (25OHD$_3$). In the kidney, 25OHD$_3$ is hydroxylated by the enzyme 25OHD$_3$-1α-hydroxylase (1α-OHase) to form 1,25-dihydroxyvitamin D_3 (1,25(OH)$_2$D$_3$), the most active vitamin D metabolite (Fig. 1). 1α-OHase expression is not restricted to the kidney. Several cell types like macrophages, osteoblasts and neurons have also been shown to express 1α-OHase (Table 1)[3–5].

The primary function of the vitamin D endocrine system is maintaining Ca and phosphate homeostasis. Vitamin D stimulates both intestinal absorption and renal reabsorption of Ca and phosphate. Vitamin D deficiency results in decreased Ca and phosphate (re)absorption and subsequently lower serum levels of Ca and phosphate. This stimulates parathyroid hormone (PTH) secretion from the parathyroid glands[6]. PTH stimulates renal 1α-OHase expression and 1,25(OH)$_2$D$_3$ formation. PTH also stimulates osteoclast formation (osteoclastogenesis). Osteoclasts stimulate bone resorption, releasing Ca and phosphate ions from the bone into the blood. A recent animal study has demonstrated that osteoclastogenesis was increased in mice when serum 25OHD$_3$ levels were < 80 nmol/l and this was positively associated with the receptor activator for NF-κB ligand/osteoprotegerin ratio. This increase in bone resorption was associated with the development of osteopenia and osteoporosis[7]. The optimal serum 25OHD$_3$ level in human subjects to prevent stimulation of osteoclastogenesis is also believed to be about 80 nmol/l[7].
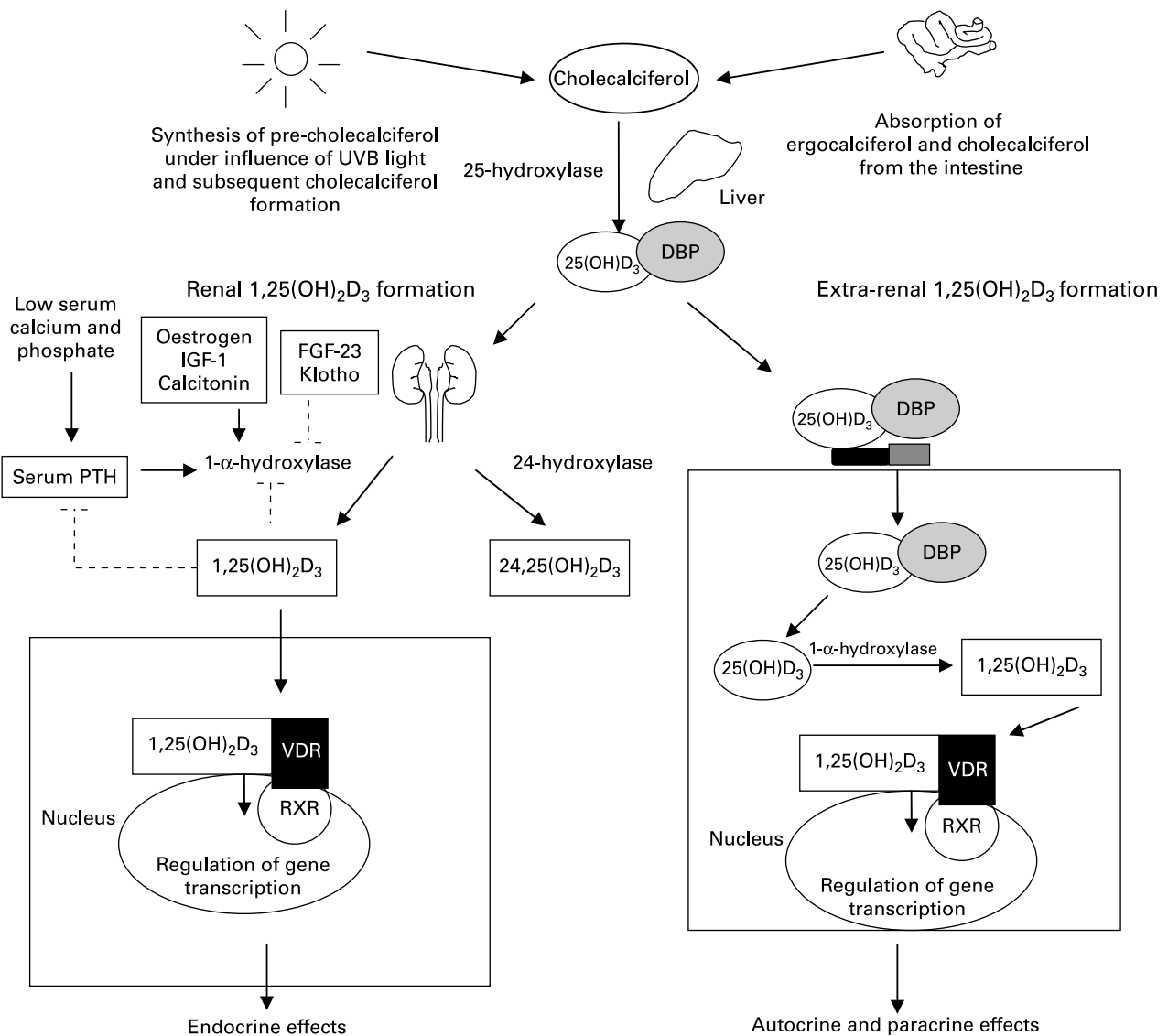

Fig. 1. Different pathways for the activation of vitamin D. Inert vitamin D precursors are either formed in the skin after exposure to UVB or derived form the diet. These precursors are hydroxylated in the liver to form 25-hydroxyvitamin D_3 (25(OH)D_3). The 25(OH)D_3 is bound to the vitamin D-binding protein (DBP). The final 1-α-hydroxylation step that forms the most active vitamin D metabolite, 1,25-dihydroxyvitamin D_3 (1,25(OH)$_2$D$_3$) occurs either in the kidney (bulk) or in the extra-renal cells expressing the 1-α-hydroxylase enzyme. The 1,25(OH)$_2$D$_3$ formation in the kidney is tightly regulated via a feedback mechanism and the active vitamin D formed in the kidney exerts endocrine effects after binding to the vitamin D receptor (VDR). The VDR forms a heterodimer with the retinoid receptor (RXR) and regulates gene transcription. The active vitamin D formed extra-renally exerts paracrine and autocrine effects. IGF, insulin-like growth factor; FGF, fibroblast growth factor; PTH, parathyroid hormone; 24,25(OH)$_2$D$_3$, 24,25-dihydroxyvitamin D_3.

Other hormones that are known to stimulate renal 1α-OHase expression are insulin-like growth factor 1, calcitonin and oestrogen[8,9]. Increases in serum Ca, phosphate and 1,25(OH)$_2$D$_3$ levels down-regulate renal 1α-OHase expression. Serum 1,25(OH)$_2$D$_3$ levels are also regulated by the enzyme 25OHD$_3$-24-hydroxylase (24OHase). Expression of 24OHase in the kidney is stimulated by 1,25(OH)$_2$D$_3$ and this enzyme converts 1,25(OH)$_2$D$_3$ into less active metabolites. These feedback mechanisms play an important role in the protection against hypercalcaemia and hyperphosphataemia[10].

Extra-renal 1α-OHase expression and activity is modulated differently from renal 1α-OHase and is less sensitive to feedback regulation by 1,25(OH)$_2$D$_3$[11]. It is suggested that induction of extra-renal 1α-OHase involves regulatory pathways that differ from the renal, cyclic AMP-mediated pathway. For example,

in osteoblasts, 1α-OHase expression and activity is not influenced by the levels of 1,25(OH)$_2$D$_3$, PTH and Ca like renal 1α-OHase. IL1β, an activator of NF-κB, stimulates both 1α-OHase expression and activity in osteoblasts[4]. In macrophages, immune signals such as TNFα and interferon-γ modulate 1α-OHase expression, while in vascular smooth muscle cells extra-renal 1α-OHase expression and activity is stimulated by the hormones PTH and oestrogen (Table 1)[12,13]. Regulators of 1α-OHase expression and activity in most extra-renal tissues and the functions of the extra-renally formed 1,25(OH)$_2$D$_3$ are still largely unknown. Some age-related effects on extra-renal 1α-OHase expression have been reported[14]. In an animal model, ageing resulted in decreased bone 1α-OHase expression[15]. Specific effects of ageing on the 1α-OHase expression and the activity in extra-renal tissues

Table 1. Extra-renal expression of the 1α-hydroxylase (1α-OHase) enzyme and effects of potential regulators relevant for ageing*

Cells and tissues expressing the 1α-OHase enzyme	Effects of potential regulators of 1α-OHase expression and/or activity
Placenta	$1,25(OH)_2D_3$: ↓ [107]
Keratinocytes	TGFβ1: ↑ [108], IFN-γ: ↑ [109], $1,25(OH)_2D_3$: – [110], TNFα: – [111], Ca: – [112], PTH: ↑ [112]
Pancreatic cells	–
Immune system (monocytes/macrophages)	$1,25(OH)_2D_3$: ↑ [113], PTH: – [113], TNFα: ↑ [114], IFN-γ: ↑ [115]
Prostate cells	$1,25(OH)_2D_3$: ↓ [116], Ca: – [116], PTH: – [116], EGF: ↑ [117], oestrogenic compounds: ↓ [118]
Osteoblasts	$1,25(OH)_2D_3$: – [4], PTH: – [4], Ca: – [4], IL 1β: ↑ [4], dihydrotestosterone: ↑ (in males) [14], oestrogenic compounds: ↑ (in females) [14]
Colon epithelium	$1,25(OH)_2D_3$: ↓ [119], TGFβ1: ↑ (?) [120], dietary Ca content: – [121]
Central nervous system	$1,25(OH)_2D_3$: ↑ [122]
Vascular endothelial cells	TNFα: ↑ [123], IFN-γ: ↑ [123]
Vascular smooth muscle cells	Oestrogenic compounds: ↑ [12], PTH: ↑ [12]
Breast tissue	$1,25(OH)_2D_3$: ↓ [124], $25OHD_3$: ↑ [125], oestrogenic compounds: ↑ [126]

*Based on *ex vivo*, *in vitro* and animal studies.
$1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; TGFβ1, transforming growth factor β1; IFN-γ, interferon-γ; PTH, parathyroid hormone; EGF, epidermal growth factor; ↑, stimulating effect; ↓, inhibiting effect; –, no effect; (?), uncertain.

and health consequences of alterations in 1α-OHase expression and activity with ageing remain to be elucidated.

In recent years, several proteins have been discovered, which are important regulators of both Ca and phosphate homeostasis and the vitamin D endocrine system. These are fibroblast growth factor-23 (FGF-23) and klotho, a β-glucuronidase[16,17]. FGF-23 is involved in the regulation of renal phosphate excretion. FGF-23 inhibits expression of the renal sodium-phosphate transporter and thereby increases phosphate excretion[18]. In addition, FGF-23 decreases renal 1α-OHase expression, stimulates 24OHase expression and decreases both PTH mRNA expression and PTH secretion from the parathyroid gland[18–20]. This leads to lower $1,25(OH)_2D_3$ levels and thus decreases vitamin D-related effects on Ca and phosphate homeostasis. Klotho is involved in the regulation of renal Ca absorption and acts as a co-receptor or cofactor for other proteins such as FGF-23[21,22]. Klotho is capable of binding to various FGF receptors and enhances FGF-23 signalling. FGF-23 and klotho thus have important functions in regulating Ca and phosphate homeostasis and are important for skeletal health, both via effects on the vitamin D endocrine system and via direct, non-vitamin D-dependent effects.

Effects of ageing on vitamin D metabolism

Vitamin D deficiency is a worldwide problem[6]. Although in Europe and the United States there has been strong attention on vitamin D in recent years and vitamin D-fortified food products are widely available, vitamin D deficiency is still very prevalent among older people[23–25]. Mean serum $25OHD_3$ concentrations in The Netherlands in independent community-dwelling older people are about 30 nmol/l and in institutionalised older people about 20–25 nmol/l[26,27]. Similar serum $25OHD_3$ levels among community-dwelling elderly have been reported in the United Kingdom and Germany[28,29]. Reports suggest that serum $25OHD_3$ levels in older people in the United States are higher than in Europe[26,30,31]. This is most likely due to higher oral intake of vitamin D in the United States where vitamin D fortification of food is more prevalent than that in Europe[24,32]. However,

even in the United States, >50% of the community-dwelling elderly are reported to have serum $25OHD_3$ levels <75 nmol/l and about 30% of the elderly have levels <50 nmol/l[30].

The high prevalence of vitamin D deficiency in older people may have several causes. Cholecalciferol synthesis in the skin after sun exposure is less effective in old age because of a decline in cutaneous levels of 7-dehydrocholesterol[33]. The level in a 70-year-old is only approximately 25% of the 7-dehydrocholesterol level in young persons[34]. This is worsened by the decreased exposure to sunlight with ageing due to immobility, lack of transport and social isolation[35,36]. Another factor contributing to the increased risk of vitamin D deficiency is an increase in body fat with ageing. The increase in fat mass leads to a larger distribution volume for the fat-soluble $25OHD_3$, which decreases the bioavailability of $25OHD_3$. Consequently, an inverse association has been demonstrated between BMI and both serum levels $25OHD_3$ and $1,25(OH)_2D_3$ and a positive association between BMI and PTH levels has been demonstrated[37,38].

An age-related decrease in $1,25(OH)_2D_3$ levels has also been suggested but reports are conflicting[39]. When vitamin D levels are low, compensatory hyperparathyroidism increases renal conversion of $25OHD_3$ to $1,25(OH)_2D_3$ and thereby maintains normal or even slightly elevated levels of this metabolite. As vitamin D deficiency worsens, $1,25(OH)_2D_3$ formation is impaired due to a lack of substrate[39]. Additionally, several age-related effects have been reported that could lead to lower $1,25(OH)_2D_3$ levels with ageing. First, renal function declines with age and this is accompanied with a decline in renal 1α-OHase activity and thus impaired conversion of $25OHD_3$ to $1,25(OH)_2D_3$[40]. Second, levels of insulin-like growth factor 1, calcitonin and oestrogen, which stimulate 1α-OHase expression and activity, decrease with ageing[41]. Furthermore, $1,25(OH)_2D_3$ metabolism may increase with ageing. In an animal model, an age-dependent increase in renal 24OHase expression was reported. This occurred predominantly in female animals, suggesting an effect of ovarian hormones[42]. Ovariectomy in these animals was indeed associated with up-regulation of 24OHase expression. Interestingly, the induction of 24OHase by $1,25(OH)_2D_3$ may also be affected by ageing[43].

Effects of ageing on vitamin D action

The active metabolite $1,25(OH)_2D_3$ exerts its function via the vitamin D receptor (VDR), a nuclear receptor. Upon binding of $1,25(OH)_2D_3$, the VDR forms a heterodimer with the retinoid receptor and binds to a vitamin D-responsive element in the promoter region of a target gene. This influences transcription of vitamin D-responsive genes[1]. In addition, the functions of the VDR are not limited to the binding to vitamin D-responsive element. The VDR has also been found to bind β-catenin, a key transcriptional factor in the Wnt signalling pathway[44,45]. This pathway has been implicated in a number of malignancies. By binding to β-catenin, the VDR blocks its transcriptional activity and so exerts antiproliferative properties. Besides genomic effects via the VDR, $1,25(OH)_2D_3$ also exerts non-genomic effects via a membrane-bound plasma receptor or second messengers such as cyclic AMP. These are rapid effects that do not depend on gene transcription.

Almost all tissues and cells in the body express the VDR, including those not directly involved in the regulation of Ca homeostasis, enabling a broad range of effects. Extra-renal $1,25(OH)_2D_3$ formation in various tissues implies that $1,25(OH)_2D_3$ is also capable of exerting paracrine and autocrine effects in addition to the well-known endocrine effects. Among the paracrine and autocrine effects are regulation of cell proliferation, differentiation and apoptosis[46].

Alterations in VDR expression leading to vitamin D resistance with ageing have received particular interest. With ageing, a decrease in VDR expression in bone, intestine and muscle tissue has been reported[47–49]. Various factors are known to influence VDR expression. Oestrogen, growth hormones and $1,25(OH)_2D_3$ are stimulators of VDR expression but serum levels decrease with ageing[50,51]. On the other hand, TNFα has been shown to down-regulate VDR expression, while serum TNFα levels increase with ageing[52,53].

In addition to a decrease in VDR numbers, binding of $1,25(OH)_2D_3$ to the VDR might also be decreased with ageing. A recent animal study, using competition VDR-binding assays with $[^3H]-1,25(OH)_2D_3$, has reported a decrease in $1,25(OH)_2D_3$ binding to the VDR with ageing in duodenal tissue[54]. Whether this also occurs in human subjects is not known.

Parathyroid hormone and ageing

Like $25OHD_3$, PTH levels also exhibit seasonal variation with the highest PTH levels observed during the winter months[55]. A (secondary) rise in PTH levels is generally observed with ageing with a prevalence varying from 20 to 60 %[56]. The most important causes of this secondary rise with ageing are vitamin D deficiency and resistance, renal insufficiency and low dietary intake of Ca[57]. PTH stimulates $1,25(OH)_2D_3$ formation and mobilises Ca from bone in order to maintain normal serum Ca levels[1]. Hyperparathyroidism not only negatively influences bone health but also is associated with sarcopaenia and falls as PTH stimulates muscle protein breakdown[58]. Furthermore, hyperparathyroidism has been related to cardiovascular events as PTH has been shown to promote vascular calcification[59]. Recently, elevated PTH levels have been shown to be an independent predictor of impaired long-term survival prognosis in older people[56]. High serum PTH levels (≥ 63 ng/l) were associated with significant increases in mortality (hazard ratio $= 1.56$, 95 % CI: 1.29, 1.88) and a 2.3-year reduction of median life expectancy in a cohort of older patients[56].

Calcium homeostasis and ageing

Ca homeostasis involves a coordinated control of Ca handling by the intestine, kidney and bone under the influence of primarily PTH and $1,25(OH)_2D_3$. Ageing, vitamin D deficiency and vitamin D resistance all affect these processes negatively. The two main mechanisms for Ca (re)absorption are a transcellular (active) and a paracellular (passive) route. The transcellular route involves entry of Ca into the cell at the apical side of the cell via Ca channels, diffusion of Ca through the cytosol bound to calbindins and active extrusion of Ca across the basolateral membrane via a Ca pump or a Na/Ca exchanger[57]. The epithelial Ca channels are members of the transient receptor potential (TRP) super family and more precisely, the vanilloid subfamily (TRPV). The TRPV5 channel is the major isoform in the kidney, while the TRPV6 channel is highly expressed in the intestine. The paracellular route involves diffusion of Ca via tight junctions between epithelial cells.

Ageing and intestinal calcium absorption

An age-related decrease in intestinal Ca absorption has long been recognised[60]. In the search for age-related factors that explain this decrease in absorption, attention has focused on TRPV6. A TRPV6 mouse knockout model illustrated the importance of TRPV6 for intestinal Ca absorption. In TRPV6 knockout mice, intestinal Ca absorption was decreased by 60 %[61]. Both in animal models and in human subjects, intestinal TRPV6 expression shows an age-dependent decline[48]. This is probably due to several effects as TRPV6 expression is regulated by $1,25(OH)_2D_3$, oestrogen, PTH and dietary Ca intake[57]. Recently, animal models have shed light on the importance of vitamin D metabolites for TRPV6 expression. Both in VDR and in 1α-OHase knockout mice, intestinal TRPV6 expression is strongly reduced, which impairs intestinal Ca absorption[62,63]. In addition, the ability of vitamin D metabolites to stimulate intestinal TRPV6 expression also seems to decrease with ageing[64].

The effects of ageing on TRPV6 expression differ among men and women. A recent study has reported that duodenal TRPV6 expression in both young and old men is strongly correlated with vitamin D status[48]. In women, however, TRPV6 expression decreased with ageing but no correlation was found with vitamin D status. In women, there was an age-dependent decline in VDR expression in the duodenal biopsies that was not found in men, which could account for the reduced vitamin D responsiveness and thus lower TRPV6 expression in women[48]. A possible explanation for decreased VDR expression could be decreased oestrogen levels with ageing. Oestrogen is important for vitamin D responsiveness as it stimulates both VDR and TRPV6 expressions[57]. Although the strongest decline in intestinal Ca absorption is seen after the menopause due to decreasing serum levels of oestrogen, another late age-related decrease in intestinal Ca absorption,

in addition to the decline that occurs at the menopause, has also been reported in women after the age of 75[65]. This decrease in intestinal Ca absorption of nearly 30 % was independent of serum levels of $1,25(OH)_2D_3$ and $25OHD_3$ and of renal function. The cause of this late decline in Ca absorption, which is most likely due to increased vitamin D resistance, remains to be clarified.

In men, the importance of the sex hormone testosterone for Ca absorption is not well known and remains to be studied. A stimulating effect of testosterone on TRPV6 expression has been suggested[66].

Ageing and renal calcium reabsorption

Less is known about the TRPV5 Ca channel. Like TRPV6, an age-related decrease in TRPV5 expression has been reported[67]. Expression of TRPV5 is mainly regulated by $1,25(OH)_2D_3$, PTH and klotho[16,68]. Klotho is important for TRPV5 expression as it cleaves a carbohydrate residue from the Ca channel TRPV5, which increases TRPV5 expression and activity by trapping it in the plasma membrane[16]. Expression of klotho itself is positively regulated by $1,25(OH)_2D_3$ and oestrogen[69]. Several recent reports have demonstrated that klotho expression decreases with ageing[70,71]. In linking klotho expression to renal Ca absorption, it has been speculated that klotho deficiency may result in the down-regulation of TRPV5 expression and thus impairment of renal Ca reabsorption[72]. The importance of TRPV5 for renal Ca reabsorption has recently been demonstrated. TRPV5 knockout mice have severe hypercalciuria and decreased serum Ca levels[73]. Klotho knockout mice exhibit both decreased renal TRPV5 expression and decreased renal Ca reabsorption[74].

As serum Ca levels normally fluctuate between narrow margins, interplay between intestinal Ca absorption and renal reabsorption is required. A decrease in renal Ca reabsorptive capability is compensated for by an increase in intestinal absorption. A recent animal model has demonstrated that TRPV5 expression is an important determinant of TRPV6 expression. TRPV5 knockout mice have an increased intestinal TRPV6 expression and thus increased rate of intestinal Ca absorption[67]. In double TRPV5 and 1α-OHase knockout mice, the up-regulation of intestinal Ca transport was abolished suggesting that this is a vitamin D-dependent effect[75]. In patients with idiopathic hypercalciuria, a disease state characterised by decreased renal Ca absorption and high urine levels of Ca, a compensatory increase in $1,25(OH)_2D_3$ levels and intestinal Ca absorption is frequently observed[76]. The relevance of this interplay for maintaining Ca homeostasis in older people and effects of ageing remain to be studied.

Ageing and calbindins

Calbindins are cytosolic Ca-binding proteins. There are two major subclasses of calbindins: calbindin-D9k, which predominantly co-localises with TRPV6 in the small intestine, and calbindin-28k, which predominantly co-localises with TRPV5 in the kidney[57]. Calbindins act to facilitate the diffusion of Ca through the cell interior towards the basolateral membrane. By buffering Ca, calbindins protect cells against toxic effects during states of high Ca influx.

Anti-apoptotic effects of calbindins have been reported in different tissues such as neurons, osteoblasts and pancreatic β cells[10]. Calbindin expression decreases with ageing, which could contribute to decreased Ca (re)absorption with ageing due to impaired transcellular diffusion[42]. This is also influenced by vitamin D deficiency as vitamin D stimulates calbindin expression in both the intestine and the kidney[10].

Other age-related effects on calcium absorption

PTH, besides stimulating intestinal Ca absorption via stimulation of renal 1α-OHase activity and thus $1,25(OH)_2D_3$ formation and subsequently TRPV5 and TRPV6 expressions, also has direct effects on Ca absorption. The stimulation of duodenal Ca uptake by PTH has been demonstrated in an animal model[77]. In rat enterocytes, PTH enhances Ca influx through activation of the voltage-gated apical Ca channels and the cyclic AMP second messenger system. Interestingly, in aged duodenal cells, PTH is more efficient in stimulating Ca absorption when compared with duodenal cells of young rats[78]. This is most likely due to alterations in signal transduction via the PTH receptor that occur with ageing. It has been speculated that this increased efficiency is a compensatory mechanism in older people in states of impaired vitamin D status[57].

Another determinant of Ca absorption is the bioavailability of dietary Ca itself. Low-Ca diets increase the efficiency of intestinal Ca absorption. The activities of all known genes involved in the transcellular pathway are enhanced by low-Ca diets, probably via activation of the vitamin D endocrine system[57].

What is vitamin D deficiency?

Measurement of serum $25OHD_3$ level is the best clinical indicator to assess vitamin D status. Serum $25OHD_3$ levels represent the combined contribution of both cutaneous synthesis and oral intake of the various dietary sources of vitamin D[79]. Levels of $1,25(OH)_2D_3$ are less suitable to assess vitamin D status because even in a state of vitamin D deficiency $1,25(OH)_2D_3$ levels can be normal or slightly elevated.

With the ever-increasing insights into the effects of vitamin D, optimal vitamin D status is becoming more difficult to define. Criteria and cut-off values for vitamin D deficiency have mostly been linked to the effects of PTH levels on bone turnover. Serum $25OHD_3$ levels are inversely associated with PTH levels until an inflection point is reached. At this point, PTH levels begin to level off. Estimates for the serum $25OHD_3$ concentration at which the PTH concentration becomes constant vary from 25 to 122 nmol/l[80,81]. This wide variation in estimates is due to inter-individual variation in Ca (re)absorption and vitamin D responsiveness, as previously discussed. Old people generally require higher serum $25OHD_3$ levels, and thus vitamin D intake, to suppress PTH levels when compared with younger individuals. The capacity of the different vitamin D metabolites to raise serum $25OHD_3$ levels is unaltered with ageing[82].

When the effects on PTH levels and bone turnover are evaluated, serum $25OHD_3$ concentrations of >50 nmol/l are regarded by many as sufficient[83]. However, when other health benefits of vitamin D are taken into account, including its non-calcaemic effects, serum $25OHD_3$ concentrations of

> 75 nmol/l are advised[84]. In addition, the process of extra-renal $1,25(OH)_2D_3$ formation and autocrine and paracrine effects are most efficient when serum $25OHD_3$ levels are > 75 nmol/l[6].

In many trials that study the effect of vitamin D supplementation, Ca intake is not measured, which complicates the comparison of individual trial results. Dietary Ca content has been shown to modulate the $25OHD_3$/PTH association[81]. As Ca intake is lower, higher $25OHD_3$ serum levels are required to normalise PTH concentrations. In part, this may also explain discordant results between intervention trials with vitamin D, as Ca intake differs among countries[85,86].

Consequences of vitamin D deficiency and resistance

Vitamin D deficiency and resistance have important consequences for older people (Fig. 2). To illustrate its importance, vitamin D deficiency is associated with an increased risk for nursing home admission. The hazard ratio of nursing home admission after 6 years of follow-up for vitamin D-deficient individuals ($25OHD_3 < 25$ nmol/l) in a large cohort of older people was 3·48 (1·39–8·75) when compared with individuals with a high serum $25OHD_3$ level. The hazard ratio for vitamin D-insufficient individuals ($25OHD_3 = 25–49$ nmol/l) was 2·77 (1·17–6·55)[87]. The effects of vitamin D on bone, intestine and kidney, which are regarded as the classical target tissues, have been the subject of many studies for a long period of time. However, as the VDR is being found in increasingly more tissues, implications of vitamin D in many different disease states are being reported due to the

effects of vitamin D outside these classical target tissues (Fig. 2). Detailed effects of vitamin D have been reported on cardiovascular health, immune system, neurological diseases and cancer. The discussion of the effects of vitamin D in these disease states is beyond the scope of the present paper, but excellent reviews have recently been published[3,5,88,89]. Of note, recently adipose tissue has been shown to be a target tissue of vitamin D[90]. With ageing, there is an accumulation of fat in bone marrow at the expense of osteoblastogenesis, contributing to the development of senile osteoporosis. Vitamin D has been shown to block adipogenesis by inhibiting the expression of PPARγ2, a critical transcription factor for adipogenesis in bone marrow[90].

In general, the advancing knowledge of the effects of vitamin D in all these tissues further strengthens the call for adequate treatment of vitamin D deficiency[91].

Treatment of vitamin D deficiency

Given the high prevalence of vitamin D deficiency in old age and the severe health consequences, a proactive approach from clinicians to case finding and adequate treatment of vitamin D deficiency is needed. Important considerations besides age are sex, BMI, skin colour, mobility, housing and dietary intake of both Ca and vitamin D[35,92,93]. Giving individualised treatment advice is complicated by the fact that the ideal vitamin D level has not yet been defined and the treatment effect on, for example, secondary hyperparathyroidism is also dependent on the dietary intake of Ca, which shows
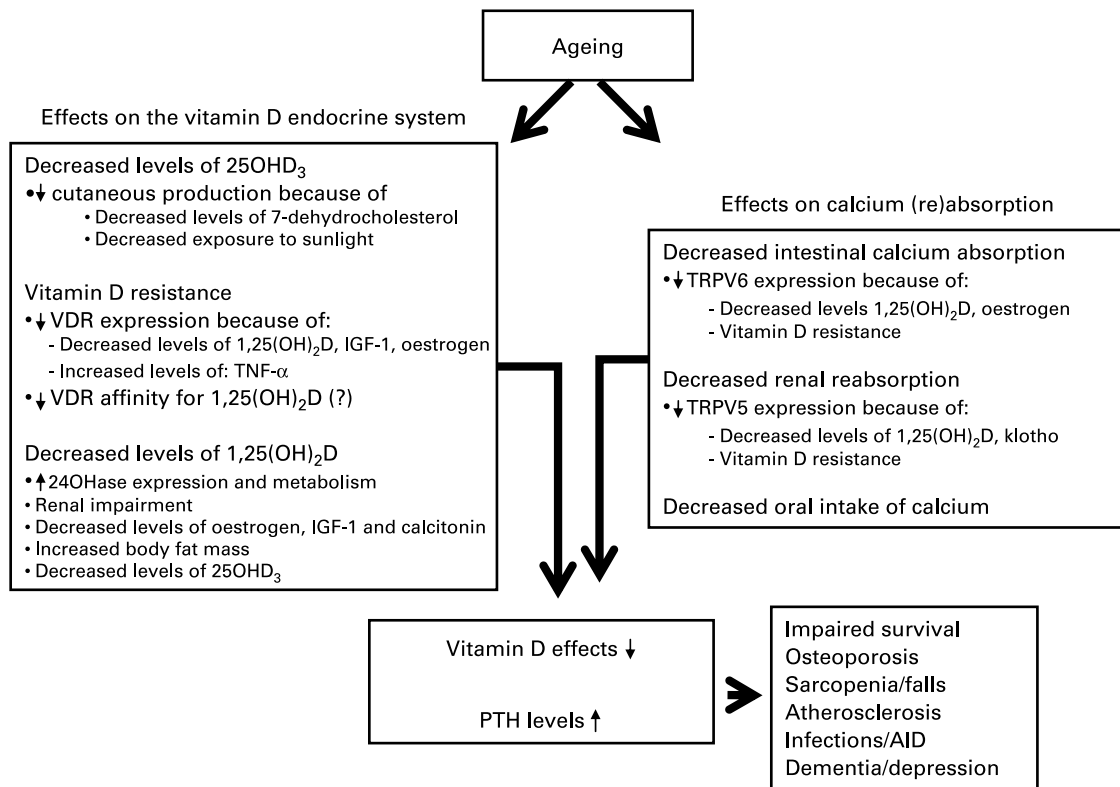

Fig. 2. Consequences of ageing on both vitamin D endocrine system and calcium absorption. $25OHD_3$, 25-hydroxyvitamin D_3; VDR, vitamin D receptor; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; IGF, insulin-like growth factor; 24OHase, 24-hydroxylase; TRPV, transient receptor potential vanilloid; AID, auto-immune disorder.

regional differences. In general, mobile, Caucasian community-dwelling elderly, who have a varied diet, need vitamin D supplementation of 10–20 μg (400 IU–800 IU)/d to reach serum vitamin D levels of 50–75 nmol/l. Frail or institutionalised elderly on the other hand are suggested to need up to 50 μg (2000 IU)/d[84,94]. The effectiveness of this high-dose vitamin D supplementation in raising serum 25OHD₃ levels adequately has been demonstrated in several clinical trials[95–97]. However, robust evidence on the optimal dose of vitamin D supplementation in specific high-risk groups is still lacking. In a recent report by the Dutch Health Council[98], 20 μg (800 IU) daily is advised for high-risk groups, i.e., persons with osteoporosis, residents of care homes, women aged 50 + and men aged 70 + with dark skin colour or housebound individuals.

Oral supplementation is the most effective intervention to treat vitamin D deficiency. Ergocalciferol is equally as effective as cholecalciferol in raising serum 25OHD₃ levels[99]. Daily dosing is the most efficient interval to raise serum 25OHD₃ concentrations when compared with weekly or monthly administration[26].

Although vitamin D supplementation therapy is generally regarded as safe, cases of iatrogenic and accidental overdose with cholecalciferol have been reported[100,101]. Most safety data concerning the use of high-dose cholecalciferol supplementation come from observations in relatively young individuals. Few studies have used high-dose cholecalciferol supplementation for longer periods in frail, older patients. Frail old people, particularly the institutionalised, often have poor daily fluid intake, use diuretics and have less thirst sensation than younger persons.

The need for high-dose supplementation therapy on the one hand, and the increased risk of dehydration on the other hand, may potentially increase the risk of accidental hypercalcaemia in these patients.

Recently, concerns have risen regarding the possible negative health effects of vitamin D supplementation[88]. The recent discovery of FGF-23 and klotho has given more insight into possible negative health effects of vitamin D supplementation and hypervitaminosis D. In animal studies, both FGF-23 and klotho knockout mice have increased expression of the enzyme 1α-OHase. These mice have increased serum levels of 1,25(OH)₂D₃, Ca and phosphate and have overall an identical phenotype. These knockout mice, despite their high levels of 1,25(OH)₂D₃, develop osteoporosis, vascular and soft tissue calcifications, muscle wasting, pulmonary emphysema and have a shortened lifespan[102,103]. Klotho knockout mice have very high levels of FGF-23 (about 2000-fold higher), but have no sign of phosphaturia, illustrating the importance of klotho for FGF-23 signalling[18,21]. Normalisation of vitamin D activity in both klotho and FGF-23 knockout mice by either feeding them a vitamin D-deficient diet or knockout of the 1α-OHase enzyme increased survival and rescued most of the phenotype, illustrating that these effects are indeed vitamin D related[102,104]. Similar effects of hypervitaminosis D due to accidental overdose in human subjects have been reported[101,105]. Discontinuation of vitamin D supplementation in human subjects with hypervitaminosis D due to excessive intake of vitamin D resulted in normalisation of serum levels of 25OHD₃, gradual recovery of bone density mineral and normalisation of the ratio of urinary Ca to creatinine[106]. So, when a patient is suffering from osteoporosis, clinicians should also consider, although rare, the possibility of vitamin D overdose.

Conclusions

Vitamin D is a pleiotropic hormone. Besides the effects on classical tissues like bone and intestine, vitamin D has an effect on many more tissues. Effects of vitamin D metabolites can occur via endocrine, paracrine or autocrine mechanisms.

Ageing increases the risk of vitamin D deficiency and is associated with vitamin D resistance and less efficient intestinal Ca absorption and renal reabsorption. Vitamin D supplementation doses needed to treat vitamin D deficiency and secondary hyperparathyroidism vary considerably between individuals. This makes it necessary for clinicians to give tailored advice to patients when treating hypovitaminosis D, taking into account these age-related effects and other characteristics that influence vitamin D status and Ca homeostasis. All clinicians who frequently treat older patients should take a proactive approach to screening at-risk individuals for vitamin D deficiency, as this condition is still very prevalent. When treating patients for vitamin D deficiency, Ca intake should be assessed. Possible unwanted effects of long-term vitamin D supplementation and the effects of hypervitaminosis D should be studied in forthcoming trials.

References

1. Lips P (2006) Vitamin D physiology. *Prog Biophys Mol Biol* **92**, 4–8.
2. Lips P (2007) Vitamin D status and nutrition in Europe and Asia. *J Steroid Biochem Mol Biol* **103**, 620–625.
3. Hewison M (2008) Vitamin D and innate immunity. *Curr Opin Investig Drugs* **9**, 485–490.
4. van Driel M, Koedam M, Buurman CJ, *et al.* (2006) Evidence for auto/paracrine actions of vitamin D in bone: 1alpha-hydroxylase expression and activity in human bone cells. *FASEB J* **20**, 2417–2419.
5. McCann JC & Ames BN (2008) Is there convincing biological or behavioral evidence linking vitamin D deficiency to brain dysfunction? *FASEB J* **22**, 982–1001.
6. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**, 266–281.
7. Anderson PH, Sawyer RK, Moore AJ, *et al.* (2008) Vitamin D depletion induces RANKL-mediated osteoclastogenesis and bone loss in a rodent model. *J Bone Miner Res* **23**, 1789–1797.

8. Gomez JM (2006) The role of insulin-like growth factor I components in the regulation of vitamin D. *Curr Pharm Biotechnol* **7**, 125–132.

9. Gallagher JC, Riggs BL & DeLuca HF (1980) Effect of estrogen on calcium absorption and serum vitamin D metabolites in postmenopausal osteoporosis. *J Clin Endocrinol Metab* **51**, 1359–1364.

10. Christakos S, Dhawan P, Benn B, *et al.* (2007) Vitamin D: molecular mechanism of action. *Ann N Y Acad Sci* **1116**, 340–348.

11. Hewison M, Zehnder D, Bland R, *et al.* (2000) 1alpha-Hydroxylase and the action of vitamin D. *J Mol Endocrinol* **25**, 141–148.

12. Somjen D, Weisman Y, Kohen F, *et al.* (2005) 25-Hydroxyvitamin D3-1alpha-hydroxylase is expressed in human vascular smooth muscle cells and is upregulated by parathyroid hormone and estrogenic compounds. *Circulation* **111**, 1666–1671.

13. Zehnder D, Bland R, Williams MC, *et al.* (2001) Extrarenal expression of 25-hydroxyvitamin D(3)-1 alpha-hydroxylase. *J Clin Endocrinol Metab* **86**, 888–894.

14. Somjen D, Katzburg S, Stern N, *et al.* (2007) 25 Hydroxy-vitamin D(3)-1alpha hydroxylase expression and activity in cultured human osteoblasts and their modulation by parathyroid hormone, estrogenic compounds and dihydrotestosterone. *J Steroid Biochem Mol Biol* **107**, 238–244.

15. Anderson PH, O'Loughlin PD, May BK, *et al.* (2005) Modulation of CYP27B1 and CYP24 mRNA expression in bone is independent of circulating 1,25(OH)2D3 levels. *Bone* **36**, 654–662.

16. Chang Q, Hoefs S, van der Kemp AW, *et al.* (2005) The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science* **310**, 490–493.

17. Yamashita T, Yoshioka M & Itoh N (2000) Identification of a novel fibroblast growth factor, FGF-23, preferentially expressed in the ventrolateral thalamic nucleus of the brain. *Biochem Biophys Res Commun* **277**, 494–498.

18. Lanske B & Razzaque MS (2007) Mineral metabolism and aging: the fibroblast growth factor 23 enigma. *Curr Opin Nephrol Hypertens* **16**, 311–318.

19. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V, *et al.* (2007) The parathyroid is a target organ for FGF23 in rats. *J Clin Invest* **117**, 4003–4008.

20. Shimada T, Hasegawa H, Yamazaki Y, *et al.* (2004) FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res* **19**, 429–435.

21. Urakawa I, Yamazaki Y, Shimada T, *et al.* (2006) Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature* **444**, 770–774.

22. Nabeshima Y & Imura H (2008) alpha-Klotho: a regulator that integrates calcium homeostasis. *Am J Nephrol* **28**, 455–464.

23. Lanske B & Razzaque MS (2007) Vitamin D and aging: old concepts and new insights. *J Nutr Biochem* **18**, 771–777.

24. Holden JM, Lemar LE & Exler J (2008) Vitamin D in foods: development of the US Department of Agriculture database. *Am J Clin Nutr* **87**, 1092S–1096S.

25. Yetley EA (2008) Assessing the vitamin D status of the US population. *Am J Clin Nutr* **88**, 558S–564S.

26. Chel V, Wijnhoven HA, Smit JH, *et al.* (2008) Efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. *Osteoporos Int* **19**, 663–671.

27. Lips P (2001) Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocr Rev* **22**, 477–501.

28. Gallacher SJ, McQuillian C, Harkness M, *et al.* (2005) Prevalence of vitamin D inadequacy in Scottish adults with non-vertebral fragility fractures. *Curr Med Res Opin* **21**, 1355–1361.

29. Hintzpeter B, Mensink GB, Thierfelder W, *et al.* (2008) Vitamin D status and health correlates among German adults. *Eur J Clin Nutr* **62**, 1079–1089.

30. Holick MF, Siris ES, Binkley N, *et al.* (2005) Prevalence of vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. *J Clin Endocrinol Metab* **90**, 3215–3224.

31. Kuriacose R & Olive KE (2008) Prevalence of vitamin D deficiency and insufficiency in northeast Tennessee. *South Med J* **101**, 906–909.

32. Calvo MS, Whiting SJ & Barton CN (2005) Vitamin D intake: a global perspective of current status. *J Nutr* **135**, 310–316.

33. MacLaughlin J & Holick MF (1985) Aging decreases the capacity of human skin to produce vitamin D3. *J Clin Invest* **76**, 1536–1538.

34. Holick MF & Chen TC (2008) Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* **87**, 1080S–1086S.

35. Holick MF, Matsuoka LY & Wortsman J (1989) Age, vitamin D, and solar ultraviolet. *Lancet* **ii**, 1104–1105.

36. Adami S, Giannini S, Bianchi G *et al.* (2008) Vitamin D status and response to treatment in post-menopausal osteoporosis. *Osteoporos Int* (Epublication ahead of print version).

37. Konradsen S, Ag H, Lindberg F, *et al.* (2008) Serum 1,25-dihydroxy vitamin D is inversely associated with body mass index. *Eur J Nutr* **47**, 87–91.

38. Snijder MB, van Dam RM, Visser M, *et al.* (2005) Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab* **90**, 4119–4123.

39. Lau KH & Baylink DJ (1999) Vitamin D therapy of osteoporosis: plain vitamin D therapy versus active vitamin D analog (D-hormone) therapy. *Calcif Tissue Int* **65**, 295–306.

40. Dukas LC, Schacht E, Mazor Z, *et al.* (2005) A new significant and independent risk factor for falls in elderly men and women: a low creatinine clearance of less than 65 ml/min. *Osteoporos Int* **16**, 332–338.

41. Thorner MO & Nass R (2007) Human studies of growth hormone and aging. *Pediatr Endocrinol Rev* **4**, 233–234.

42. Matkovits T & Christakos S (1995) Variable *in vivo* regulation of rat vitamin D-dependent genes (osteopontin, Ca,Mg-adenosine triphosphatase, and 25-hydroxyvitamin D3 24-hydroxylase): implications for differing mechanisms of regulation and involvement of multiple factors. *Endocrinology* **136**, 3971–3982.

43. Armbrecht HJ & Boltz MA (1991) Expression of 25-hydroxyvitamin D 24-hydroxylase cytochrome P450 in kidney and intestine. Effect of 1,25-dihydroxyvitamin D and age. *FEBS Lett* **292**, 17–20.

44. Bikle DD (2007) What is new in vitamin D: 2006–2007. *Curr Opin Rheumatol* **19**, 383–388.

45. Shah S, Islam MN, Dakshanamurthy S, *et al.* (2006) The molecular basis of vitamin D receptor and beta-catenin crossregulation. *Mol Cell* **21**, 799–809.

46. van Driel M, Koedam M, Buurman CJ, *et al.* (2006) Evidence that both 1alpha,25-dihydroxyvitamin D3 and 24-hydroxylated D3 enhance human osteoblast differentiation and mineralization. *J Cell Biochem* **99**, 922–935.

47. Duque G, El Abdaimi K, Macoritto M, *et al.* (2002) Estrogens (E2) regulate expression and response of 1,25-dihydroxyvitamin D3 receptors in bone cells: changes with aging and hormone deprivation. *Biochem Biophys Res Commun* **299**, 446–454.

48. Walters JR, Balesaria S, Chavele KM, *et al.* (2006) Calcium channel TRPV6 expression in human duodenum: different relationships to the vitamin D system and aging in men and women. *J Bone Miner Res* **21**, 1770–1777.

49. Bischoff-Ferrari HA, Borchers M, Gudat F, et al. (2004) Vitamin D receptor expression in human muscle tissue decreases with age. J Bone Miner Res 19, 265–269.

50. Welsh J, Wietzke JA, Zinser GM, et al. (2002) Impact of the Vitamin D3 receptor on growth-regulatory pathways in mammary gland and breast cancer. J Steroid Biochem Mol Biol 83, 85–92.

51. Klaus G, Weber L, Rodriguez J, et al. (1998) Interaction of IGF-I and 1 alpha, 25(OH)2D3 on receptor expression and growth stimulation in rat growth plate chondrocytes. Kidney Int 53, 1152–1161.

52. Andress D (2007) Nonclassical aspects of differential vitamin D receptor activation: implications for survival in patients with chronic kidney disease. Drugs 67, 1999–2012.

53. Fernandez-Martin JL, Kurian S, Farmer P, et al. (1998) Tumor necrosis factor activates a nuclear inhibitor of vitamin D and retinoid-X receptors. Mol Cell Endocrinol 141, 65–72.

54. Gonzalez Pardo V, Boland R & de Boland AR (2008) Vitamin D receptor levels and binding are reduced in aged rat intestinal subcellular fractions. Biogerontology 9, 109–118.

55. Vecino-Vecino C, Gratton M, Kremer R, et al. (2006) Seasonal variance in serum levels of vitamin D determines a compensatory response by parathyroid hormone: study in an ambulatory elderly population in Quebec. Gerontology 52, 33–39.

56. Bjorkman MP, Sorva AJ & Tilvis RS (2008) Elevated serum parathyroid hormone predicts impaired survival prognosis in a general aged population. Eur J Endocrinol 158, 749–753.

57. Perez AV, Picotto G, Carpentieri AR, et al. (2008) Minireview on regulation of intestinal calcium absorption. Emphasis on molecular mechanisms of transcellular pathway. Digestion 77, 22–34.

58. Visser M, Deeg DJ & Lips P (2003) Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. J Clin Endocrinol Metab 88, 5766–5772.

59. Rashid G, Bernheim J, Green J, et al. (2007) Parathyroid hormone stimulates endothelial expression of atherosclerotic parameters through protein kinase pathways. Am J Physiol Renal Physiol 292, F1215–F1218.

60. Bullamore JR, Wilkinson R, Gallagher JC, et al. (1970) Effect of age on calcium absorption. Lancet ii, 535–537.

61. Bianco SD, Peng JB, Takanaga H, et al. (2007) Marked disturbance of calcium homeostasis in mice with targeted disruption of the Trpv6 calcium channel gene. J Bone Miner Res 22, 274–285.

62. Hoenderop JG, van der Kemp AW, Urben CM, et al. (2004) Effects of vitamin D compounds on renal and intestinal Ca2 + transport proteins in 25-hydroxyvitamin D3-1alpha-hydroxylase knockout mice. Kidney Int 66, 1082–1089.

63. Song Y, Kato S & Fleet JC (2003) Vitamin D receptor (VDR) knockout mice reveal VDR-independent regulation of intestinal calcium absorption and ECaC2 and calbindin D9k mRNA. J Nutr 133, 374–380.

64. Armbrecht HJ, Boltz MA & Kumar VB (1999) Intestinal plasma membrane calcium pump protein and its induction by 1,25(OH)(2)D(3) decrease with age. Am J Physiol 277, G41–G47.

65. Nordin BE, Need AG, Morris HA, et al. (2004) Effect of age on calcium absorption in postmenopausal women. Am J Clin Nutr 80, 998–1002.

66. Scopacasa F, Wishart JM, Horowitz M, et al. (2004) Relation between calcium absorption and serum calcitriol in normal men: evidence for age-related intestinal resistance to calcitriol. Eur J Clin Nutr 58, 264–269.

67. van Abel M, Huybers S, Hoenderop JG, et al. (2006) Age-dependent alterations in Ca2 + homeostasis: role of TRPV5 and TRPV6. Am J Physiol Renal Physiol 291, F1177–F1183.

68. Hoenderop JG, Nilius B & Bindels RJ (2005) Calcium absorption across epithelia. Physiol Rev 85, 373–422.

69. Lewin E & Olgaard K (2006) Klotho, an important new factor for the activity of Ca^{2+} channels, connecting calcium homeostasis, ageing and uraemia. Nephrol Dial Transplant 21, 1770–1772.

70. Witkowski JM, Soroczynska-Cybula M, Bryl E, et al. (2007) Klotho – a common link in physiological and rheumatoid arthritis-related aging of human $CD4^+$ lymphocytes. J Immunol 178, 771–777.

71. Duce JA, Podvin S, Hollander W, et al. (2008) Gene profile analysis implicates klotho as an important contributor to aging changes in brain white matter of the rhesus monkey. Glia 56, 106–117.

72. Nabeshima Y (2006) Toward a better understanding of klotho. Sci Aging Know Environ 8, pe11.

73. Hoenderop JG, van Leeuwen JP, van der Eerden BC, et al. (2003) Renal Ca2 + wasting, hyperabsorption, and reduced bone thickness in mice lacking TRPV5. J Clin Invest 112, 1906–1914.

74. Torres PU, Prie D, Molina-Bletry V, et al. (2007) Klotho: an antiaging protein involved in mineral and vitamin D metabolism. Kidney Int 71, 730–737.

75. Renkema KY, Nijenhuis T, van der Eerden BC, et al. (2005) Hypervitaminosis D mediates compensatory Ca^{2+} hyperabsorption in TRPV5 knockout mice. J Am Soc Nephrol 16, 3188–3195.

76. Worcester EM & Coe FL (2008) New insights into the pathogenesis of idiopathic hypercalciuria. Semin Nephrol 28, 120–132.

77. Russo de Boland A (2004) Age-related changes in the response of intestinal cells to parathyroid hormone. Mech Ageing Dev 125, 877–888.

78. Massheimer V, Picotto G, Boland R, et al. (2000) Effect of aging on the mechanisms of PTH-induced calcium influx in rat intestinal cells. J Cell Physiol 182, 429–437.

79. Zerwekh JE (2008) Blood biomarkers of vitamin D status. Am J Clin Nutr 87, 1087S–1091S.

80. Bjorkman M, Sorva A & Tilvis R (2008) Responses of parathyroid hormone to vitamin D supplementation: a systematic review of clinical trials. Arch Gerontol Geriatr (Epublication ahead of print version).

81. Adami S, Viapiana O, Gatti D, et al. (2008) Relationship between serum parathyroid hormone, vitamin D sufficiency, age, and calcium intake. Bone 42, 267–270.

82. Vieth R, Ladak Y & Walfish PG (2003) Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. J Clin Endocrinol Metab 88, 185–191.

83. Lips P (2004) Which circulating level of 25-hydroxyvitamin D is appropriate? J Steroid Biochem Mol Biol 89–90, 611–614.

84. Heaney RP (2005) The vitamin D requirement in health and disease. J Steroid Biochem Mol Biol 97, 13–19.

85. Boonen S, Lips P, Bouillon R, et al. (2007) Need for additional calcium to reduce the risk of hip fracture with vitamin D supplementation: evidence from a comparative metaanalysis of randomized controlled trials. J Clin Endocrinol Metab 92, 1415–1423.

86. Branca F & Valtuena S (2001) Calcium, physical activity and bone health-building bones for a stronger future. Public Health Nutr 4, 117–123.

87. Visser M, Deeg DJ, Puts MT, et al. (2006) Low serum concentrations of 25-hydroxyvitamin D in older persons and the risk of nursing home admission. Am J Clin Nutr 84, 616–622.

88. Richart T, Li Y & Staessen JA (2007) Renal versus extrarenal activation of vitamin D in relation to atherosclerosis, arterial stiffening, and hypertension. Am J Hypertens 20, 1007–1015.

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89. Giovannucci E (2008) Vitamin D status and cancer incidence and mortality. *Adv Exp Med Biol* **624**, 31–42.

90. Duque G & Troen BR (2008) Understanding the mechanisms of senile osteoporosis: new facts for a major geriatric syndrome. *J Am Geriatr Soc* **56**, 935–941.

91. Kimball S, Fuleihan Gel H & Vieth R (2008) Vitamin D: a growing perspective. *Crit Rev Clin Lab Sci* **45**, 339–414.

92. Bouillon R, Verstuyf A, Mathieu C, *et al.* (2006) Vitamin D resistance. *Best Pract Res Clin Endocrinol Metab* **20**, 627–645.

93. Semba RD, Garrett E, Johnson BA, *et al.* (2000) Vitamin D deficiency among older women with and without disability. *Am J Clin Nutr* **72**, 1529–1534.

94. Bischoff-Ferrari HA, Giovannucci E, Willett WC, *et al.* (2006) Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* **84**, 18–28.

95. Himmelstein S, Clemens TL, Rubin A, *et al.* (1990) Vitamin D supplementation in elderly nursing home residents increases 25(OH)D but not 1,25(OH)2D. *Am J Clin Nutr* **52**, 701–706.

96. Schleithoff SS, Zittermann A, Tenderich G, *et al.* (2006) Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr* **83**, 754–759.

97. Stefikova K, Chylova K, Krivosikova Z *et al.* (2004) Intensive vitamin D supplementation in the treatment of osteoporosis. *Vnitr Lek* **50**, 286–290.

98. Health Council of The Netherlands (2008) *Naar een toereikende inname van vitamine D* (Towards an adequate intake of vitamin D). The Hague publication no. 2008/15.

99. Holick MF, Biancuzzo RM, Chen TC, *et al.* (2008) Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. *J Clin Endocrinol Metab* **93**, 677–681.

100. Blank S, Scanlon KS, Sinks TH, *et al.* (1995) An outbreak of hypervitaminosis D associated with the overfortification of milk from a home-delivery dairy. *Am J Public Health* **5**, 656–659.

101. Hathcock JN, Shao A, Vieth R, *et al.* (2007) Risk assessment for vitamin D. *Am J Clin Nutr* **85**, 6–18.

102. Tsujikawa H, Kurotaki Y, Fujimori T, *et al.* (2003) Klotho, a gene related to a syndrome resembling human premature aging, functions in a negative regulatory circuit of vitamin D endocrine system. *Mol Endocrinol* **17**, 2393–2403.

103. Shimada T, Kakitani M, Yamazaki Y, *et al.* (2004) Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. *J Clin Invest* **113**, 561–568.

104. Razzaque MS & Lanske B (2006) Hypervitaminosis D and premature aging: lessons learned from Fgf23 and klotho mutant mice. *Trends Mol Med* **12**, 298–305.

105. Jacobus CH, Holick MF, Shao Q, *et al.* (1992) Hypervitaminosis D associated with drinking milk. *N Engl J Med* **326**, 1173–1177.

106. Adams JS & Lee G (1997) Gains in bone mineral density with resolution of vitamin D intoxication. *Ann Intern Med* **127**, 203–206.

107. Avila E, Diaz L, Barrera D, *et al.* (2007) Regulation of vitamin D hydroxylases gene expression by 1,25-dihydroxyvitamin D3 and cyclic AMP in cultured human syncytiotrophoblasts. *J Steroid Biochem Mol Biol* **103**, 90–96.

108. Schauber J, Dorschner RA, Coda AB, *et al.* (2007) Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. *J Clin Invest* **117**, 803–811.

109. Bikle DD, Pillai S, Gee E, *et al.* (1989) Regulation of 1,25-dihydroxyvitamin D production in human keratinocytes by interferon-gamma. *Endocrinology* **124**, 655–660.

110. Xie Z, Munson SJ, Huang N, *et al.* (2002) The mechanism of 1,25-dihydroxyvitamin D(3) autoregulation in keratinocytes. *J Biol Chem* **277**, 36987–36990.

111. Bikle DD, Pillai S, Gee E, *et al.* (1991) Tumor necrosis factor-alpha regulation of 1,25-dihydroxyvitamin D production by human keratinocytes. *Endocrinology* **129**, 33–38.

112. Bikle DD, Nemanic MK, Gee E, *et al.* (1986) 1,25-Dihydroxyvitamin D3 production by human keratinocytes. Kinetics and regulation. *J Clin Invest* **78**, 557–566.

113. Dusso AS, Finch J, Brown A, *et al.* (1991) Extrarenal production of calcitriol in normal and uremic humans. *J Clin Endocrinol Metab* **72**, 157–164.

114. Stoffels K, Overbergh L, Giulietti A, *et al.* (2006) Immune regulation of 25-hydroxyvitamin-D3-1alpha-hydroxylase in human monocytes. *J Bone Miner Res* **21**, 37–47.

115. Koeffler HP, Reichel H, Bishop JE, *et al.* (1985) gamma-Interferon stimulates production of 1,25-dihydroxyvitamin D3 by normal human macrophages. *Biochem Biophys Res Commun* **127**, 596–603.

116. Young MV, Schwartz GG, Wang L, *et al.* (2004) The prostate 25-hydroxyvitamin D-1 alpha-hydroxylase is not influenced by parathyroid hormone and calcium: implications for prostate cancer chemoprevention by vitamin D. *Carcinogenesis* **25**, 967–971.

117. Wang L, Flanagan JN, Whitlatch LW, *et al.* (2004) Regulation of 25-hydroxyvitamin D-1alpha-hydroxylase by epidermal growth factor in prostate cells. *J Steroid Biochem Mol Biol* **89–90**, 127–130.

118. Farhan H, Wahala K & Cross HS (2003) Genistein inhibits vitamin D hydroxylases CYP24 and CYP27B1 expression in prostate cells. *J Steroid Biochem Mol Biol* **84**, 423–429.

119. Theodoropoulos C, Demers C, Delvin E, *et al.* (2003) Calcitriol regulates the expression of the genes encoding the three key vitamin D3 hydroxylases and the drug-metabolizing enzyme CYP3A4 in the human fetal intestine. *Clin Endocrinol (Oxf)* **58**, 489–499.

120. Liu N, Nguyen L & Chun RF, *et al.* (2008) Altered endocrine and autocrine metabolism of vitamin D in a mouse model of gastrointestinal inflammation. *Endocrinology* (Epublication ahead of print version).

121. Kallay E, Bises G, Bajna E, *et al.* (2005) Colon-specific regulation of vitamin D hydroxylases – a possible approach for tumor prevention. *Carcinogenesis* **26**, 1581–1589.

122. Diesel B, Radermacher J, Bureik M, *et al.* (2005) Vitamin D(3) metabolism in human glioblastoma multiforme: functionality of CYP27B1 splice variants, metabolism of calcidiol, and effect of calcitriol. *Clin Cancer Res* **11**, 5370–5380.

123. Zehnder D, Bland R, Chana RS, *et al.* (2002) 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.

124. Kemmis CM, Salvador SM, Smith KM, *et al.* (2006) Human mammary epithelial cells express CYP27B1 and are growth inhibited by 25-hydroxyvitamin D-3, the major circulating form of vitamin D-3. *J Nutr* **136**, 887–892.

125. Peng X, Hawthorne M & Vaishnav A (2008) 25-Hydroxyvitamin D(3) is a natural chemopreventive agent against carcinogen induced precancerous lesions in mouse mammary gland organ culture. *Breast Cancer Res Treat* (Epublication ahead of print version).

126. Cross HS, Kallay E & Lechner D (2004) Phytoestrogens and vitamin D metabolism: a new concept for the prevention and therapy of colorectal, prostate, and mammary carcinomas. *J Nutr* **134**, 1207S–1212S.