# Review article

# Vitamin D in preventive medicine: are we ignoring the evidence?

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Vitamin D is metabolised by a hepatic 25-hydroxylase into 25-hydroxyvitamin D (25(OH)D) and by a renal 1α-hydroxylase into the vitamin D hormone calcitriol. Calcitriol receptors are present in more than thirty different tissues. Apart from the kidney, several tissues also possess the enzyme 1α-hydroxylase, which is able to use circulating 25(OH)D as a substrate. Serum levels of 25(OH)D are the best indicator to assess vitamin D deficiency, insufficiency, hypovitaminosis, adequacy, and toxicity. European children and young adults often have circulating 25(OH)D levels in the insufficiency range during wintertime. Elderly subjects have mean 25(OH)D levels in the insufficiency range throughout the year. In institutionalized subjects 25(OH)D levels are often in the deficiency range. There is now general agreement that a low vitamin D status is involved in the pathogenesis of osteoporosis. Moreover, vitamin D insufficiency can lead to a disturbed muscle function. Epidemiological data also indicate a low vitamin D status in tuberculosis, rheumatoid arthritis, multiple sclerosis, inflammatory bowel diseases, hypertension, and specific types of cancer. Some intervention trials have demonstrated that supplementation with vitamin D or its metabolites is able: (i) to reduce blood pressure in hypertensive patients; (ii) to improve blood glucose levels in diabetics; (iii) to improve symptoms of rheumatoid arthritis and multiple sclerosis. The oral dose necessary to achieve adequate serum 25(OH)D levels is probably much higher than the current recommendations of 5–15 μg/d.

**Vitamin D insufficiency: Vitamin D intoxication: Parathyroid hormone: Disease prevention**

Rickets, the clinical outcome of a severe vitamin D deficiency in infants, was endemic in Europe and North America during the 19th century and during the first two decades of the 20th century. Based on the observations that skin exposure to u.v. light as well as oral vitamin D intake could cure rickets, several very effective prevention strategies were performed. The so-called 'stossprophylaxis' was based on the administration of high amounts of vitamin D several times during infancy (Markestad *et al.* 1987). Moreover, young children were regularly exposed to artificial u.v. light. Present prophylaxes of rickets include a daily vitamin D supplement of 10 μg in Germany (Deutsche Gesellschaft für Ernährung *et al.* 2000), the UK (Department of Health, 1998), the Netherlands (Health Council of the Netherlands, 2000), Sweden (Axelsson *et al.* 1999), and Finland (National Nutrition Council, 1999). In the USA, the adequate intake for infants is 5 μg/d (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board and Institute of Medicine, 1997). Nowadays, rickets is rare in Europe and North America, but there is still a risk, especially if parents are not aware of preventive measures or neglect them (Hellebostad *et al.* 1985; Hartman, 2000).

There is now growing evidence that the adult European population is also at risk for an inadequate vitamin D status (see p. 554–555). The present review summarizes the evidence of an involvement of a low vitamin D status in the pathogenesis of several chronic diseases. Moreover, the amount of oral vitamin D intake to maintain an adequate vitamin D status is discussed.

## Vitamin D metabolism and actions

Vitamin D can be ingested orally or can be formed endogenously by the skin after exposure to u.v. B light

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(wavelength 290–315 nm). In the skin, a plateau of daily vitamin D production is reached after only 30 min of u.v. B irradiation (Holick, 1994). Increased melanin pigmentation reduces the efficiency of u.v. B-mediated vitamin D synthesis and necessitates increases in the exposure time required to maximize vitamin D formation, but does not influence the total content of daily vitamin D production. Orally ingested and endogenously formed vitamin D is transported to the liver and is there converted to 25-hydroxyvitamin D (25(OH)D) (Fig. 1). A strong regulation of this step does not exist and there is no significant storage of 25(OH)D in the liver of mammals. 25(OH)D is rapidly released by the liver into the blood, where it circulates with a biological half-life of approximately 12–19 d. In the kidney, 25(OH)D is enzymically converted to the vitamin D hormone 1,25-dihydroxyvitamin D (calcitriol). Renal synthesis of calcitriol is homeostatically controlled by parathyroid hormone (PTH). Synthesis of PTH is regulated by serum concentrations of Ca and P. 25(OH)D can also be converted by a renal 24-hydroxylase into 24,25-dihydroxyvitamin D. Circulating 24,25-dihydroxyvitamin D levels are very strongly correlated with circulating 25(OH)D levels. Circulating 25(OH)D levels are, however, approximately ten times higher than serum 24,25-dihydroxyvitamin D levels and are approximately 500–1000 times higher than serum calcitriol levels. Metabolism of ergocalciferol (vitamin $D_2$) and cholecalciferol (vitamin $D_3$) is similar. Oral vitamin $D_3$ intake results, however, in a 70 % higher serum 25(OH)D level in comparison with the same amount of vitamin $D_2$ (Trang et al. 1998).

Vitamin D metabolites are known as regulators of systemic Ca homeostasis with actions in the intestine, the kidneys, and bone. Calcitriol is on a molar basis the most potent vitamin D metabolite. Calcitriol increases both intestinal absorption of orally ingested Ca and tubular reabsorption of Ca by an active, receptor-mediated process in order to maintain physiological serum Ca levels.

Calcitriol plays not only a pivotal role in systemic Ca homeostasis but also in the intracellular Ca homeostasis of various tissues. Now it is clear that vitamin D receptors (VDR) exist in more than thirty different tissues (Table 1). Calcitriol functions as a steroid hormone that binds to a cytosolic VDR resulting in a selective demasking of the genome of the nucleolus. Polymorphisms of the VDR have been described for the endonuclease BmsI, Apa I, Taq I, and Fok I restriction sites.

The number of genes known to be regulated by calcitriol is still growing. Apart from a large number of Ca- and bone-related genes, numerous genes involved in the regulation of the cell cycle or humoral mechanisms (for example, cytokines involved in the immune or haematopoietic system) are also calcitriol-dependent. Calcitriol can be locally produced in several tissues that possess VDR and are responsive to this hormone. Consequently, for calcitriol a paracrine role apart from its Ca-regulating function has been proposed (Bouillon et al. 1998).
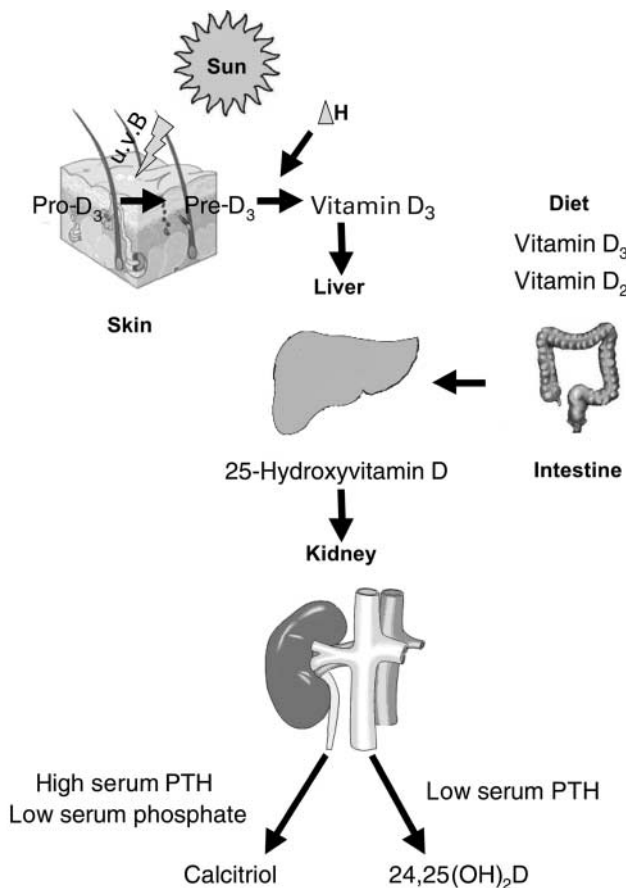


**Fig. 1.** The major metabolic pathways of vitamin D. Human sources of vitamin D are skin production of vitamin $D_3$ by u.v. light and oral intake of vitamin $D_2$ and/or vitamin $D_3$. Vitamin D is hydroxylated in the liver into 25-hydroxyvitamin D and in the kidney into the vitamin D hormone calcitriol. Renal calcitriol synthesis includes activation of $1\alpha$-hydroxylase by parathyroid hormone and suppression of the $1\alpha$-hydroxylase by high serum levels of ionized Ca. PTH; parathyroid hormone; 24,25(OH)$_2$D, 24,25-dihydroxyvitamin D.

**Table 1.** Cells with evidence for cytosolic or nuclear and/or membrane-bound vitamin D receptors (from Nemere & Farach-Carson, 1998; Norman, 1998, DeLuca & Cantorna, 2001)

| Cell type |
| --- |
| Intestinal cells |
| Muscle cells |
| Osteoblasts |
| Distal renal cells |
| Parathyroid cells |
| Islet cells, pancreas |
| Epidermal cells |
| Circulating monocytes |
| Transformed B-cells |
| Activated T-cells |
| Neurons |
| Placenta |
| Skin fibroblasts |
| Chondrocytes |
| Colon enterocytes |
| Liver cells |
| Prostate cells |
| Ovarian cells |
| Keratinocytes of skin |
| Endocrine cells, stomach |
| Aortic endothelial cells |
| Pituitary cells |

The *de novo* mRNA and protein synthesis induced by the cytosolic calcitriol–VDR complex require periods lasting hours to days. However, rapid calcitriol actions have also been observed in several tissues at both the cellular and subcellular level (Norman, 1998). These calcitriol actions cannot be explained by receptor–hormone interactions with the genome. Meanwhile, a membrane-bound VDR has been recognized in different cell lines leading to an activation of specific intracellular metabolic pathways within a few minutes. Given the pivotal role of ionized Ca in muscle contraction, nerve-impulse conduction, and other physiological phenomena, such a rapid response could be life-saving for the organism (Nemere & Farach-Carson, 1998).

There are some studies available indicating that 25(OH)D itself has important physiological functions. Dose–response studies indicate a molar potency of calcitriol relative to 25(OH)D ranging from 125:1 to 400:1 in increasing Ca absorption from the gut (Barger-Lux *et al.* 1995). Based on these molar potencies of calcitriol and 25(OH)D and the serum concentrations of the two vitamin D metabolites (approximately 1:500 to 1:1000) it can be assumed that 55 to 90 % of the circulating vitamin D activity is contributed by 25(OH)D (Barger-Lux *et al.* 1995). In line with this assumption, cross-sectional studies have demonstrated that serum 25(OH)D levels are a better indicator for intestinal Ca absorption efficiency than serum calcitriol levels (Barger-Lux *et al.* 1995; Zittermann *et al.* 1998). Consequently, very low 25(OH)D levels as found in rickets and osteomalacia result in an impaired intestinal Ca absorption leading to a severe Ca deficit in the human body. Moreover, 25(OH)D increases: (i) the uptake of $^{45}$Ca into cultured muscle cells (Selles *et al.* 1994); (ii) the intracellular Ca re-uptake into the sarcoplasmic reticulum (Poiton *et al.* 1979); (iii) the intracellular accumulation of phosphate (Birge & Haddad, 1975). Circulating

25(OH)D also serves as a substrate for the $1\alpha$-hydroxylase of various tissues that possess VDR. The tissues in the body that are not responsible for regulating extracellular Ca metabolism probably use circulating 25(OH)D to make calcitriol (Holick, 2002). Low serum 25(OH)D levels may thus impair intracellular calcitriol availability. Several tissues also possess 24-hydroxylase activity resulting in a local production of 24,25-dihydroxyvitamin D from 25(OH)D. It has been hypothesized that 24,25-dihydroxyvitamin D is indispensable for normal Ca and P homeostasis. Consequently, a cellular receptor for 24,25-dihydroxyvitamin D has been postulated by some investigators (Norman, 1998).

### Assessment of vitamin D status

Circulating 25(OH)D levels closely reflect the amount of sunlight to which the epidermis is exposed and the dietary intake of vitamin D. There is general agreement that the serum 25(OH)D level is the best indicator to define vitamin D deficiency, insufficiency, hypovitaminosis, sufficiency, and toxicity (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board and Institute of Medicine, 1997; McKenna & Freaney, 1998) (Fig. 2). Nevertheless, it is difficult to clearly define cut-off values for each stage. There is no doubt that 25(OH)D levels below 12·5 nmol/l can result in bone diseases such as rickets in infants and osteomalacia in adults (Scharla, 1998). There is, however, also evidence that 25(OH)D levels below 25 nmol/l lead to rickets and osteomalacia in the long run (Basha *et al.* 2000). Concentrations of 25(OH)D below 40–50 nmol/l reflect vitamin D insufficiency (Malabanan *et al.* 1998, Need *et al.* 2000; Vieth *et al.* 2001*b*). Values below this threshold can lead to functional alterations such as hyperparathyroidism. In subjects with 25(OH)D levels below 50 nmol/l high
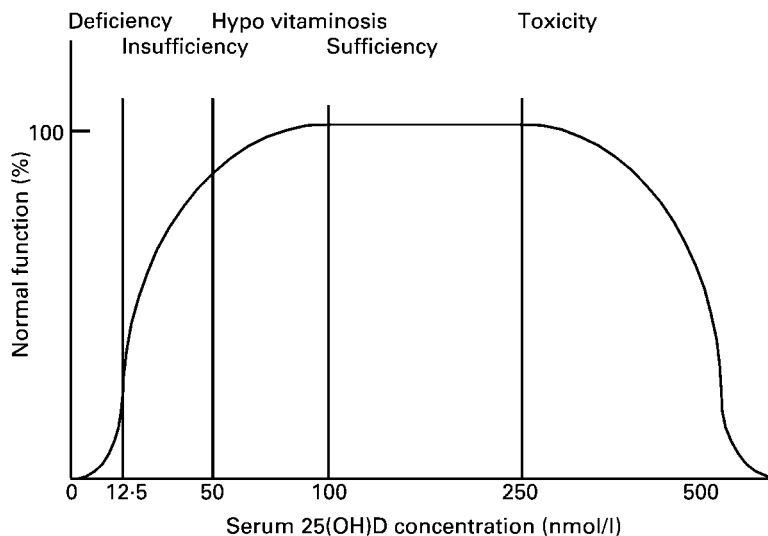


**Fig. 2.** Stages of vitamin D status. In the vitamin D deficiency range, there is severe hyperparathyroidism, Ca malabsorption, bone diseases such as rickets in infants and osteomalacia in adults, and myopathy. Vitamin D insufficiency results in mild hyperparathyroidism, low intestinal Ca absorption rates, reduced bone mineral density, and perhaps subclinical myopathy. In hypovitaminosis D, body stores of vitamin D are low and parathyroid hormone levels can be slightly elevated. In the range of vitamin D sufficiency no disturbances of vitamin D-dependent functions occur. In the vitamin D toxicity range, there is intestinal Ca hyperabsorption and increased net bone resorption leading to hypercalcaemia. 25(OH)D, 25-hydroxyvitamin D.

doses of oral vitamin D can decrease the elevated PTH levels (Malabanan et al. 1998). It must also be emphasized that oral Ca intake can suppress PTH levels (Kärkkainen et al. 1997) and oral phosphate intake can increase PTH levels (Whybro et al. 1998). These nutrients may therefore influence the vitamin D–PTH axis.

Serum calcitriol levels can be affected differently during vitamin D insufficiency or deficiency. Circulating concentrations are often similar to those of vitamin D-replete subjects (Eastwood et al. 1979). The accompanying secondary hyperparathyroidism can, however, also result in an increase in serum calcitriol levels (Adams et al. 1982; Bell et al. 1985). Moreover, even low calcitriol levels can be observed (Bouillon et al. 1987; Docio et al. 1998), most probably because of an insufficient substrate availability for the renal 1α-hydroxylase. Consequently, determination of serum calcitriol levels is not a valid measure in order to assess vitamin D status.

Serum 25(OH)D concentrations between 50 nmol/l and 80–100 nmol/l can be regarded as hypovitaminosis D, where body stores are already depleted and PTH levels can be slightly elevated, but are still in the normal range (McKenna & Freaney, 1998; Lamberg-Allardt et al. 2001).

Circulating 25(OH)D levels between 100 and 200 nmol/l can be regarded as adequate concentrations, where no disturbances in vitamin D-dependent body functions occur (Peacock, 1995). A rationale for this assumption is the observation that subjects with a constantly high u.v. B exposure living close to the equator have mean 25(OH)D levels of 107 nmol/l and upper serum levels (+2 SD) of 163 nmol/l throughout the year (Linhares et al. 1984). Moreover, American bath attendants have serum 25(OH)D levels up to 160 nmol/l (Holmes & Kummerow, 1983).

## Vitamin D status in different European population groups

In general, healthy young adults have marked seasonal fluctuations in serum 25(OH)D levels with lower concentrations in winter than in summer (Table 2). Even children and adolescents, two groups with various outdoor activities and frequent exposure to sunlight, have low 25(OH)D levels in winter (Table 2). The main reason for the low 25(OH)D levels in winter is the fact that vitamin D status is largely dependent on skin synthesis and that u.v. B radiation of the sunlight is negligible from October to April at the latitude of 52°N and from November to February at the latitude of 42°N. In contrast, skin synthesis of vitamin D is possible throughout the year at the latitude of 32°N or closer to the equator (Holick, 1994). Even premenopausal women living in a sunny country such as Turkey have very low 25(OH)D levels in summer if sufficient u.v. B irradiation of the skin is not guaranteed

**Table 2.** Vitamin D status in different European population groups during summer and winter

| Age group and country | Latitude (°North) | Mean circulating 25(OH)D level (nmol/l) | | Reference |
| | | Summer | Winter | |
|---|---|---|---|---|
| Children | | | | |
| Germany | 51 | 84 | 43 | Zittermann (1987) |
| UK: white children | 50–60 | 80 | 52 | Davies et al. (1999) |
| UK: dark-skinned children | 50–60 | | 36–42* | Lawson et al. (1999) |
| Spain | 43·5 | 75 | 32 | Docio et al. (1998) |
| Brazil | 8 (South) | 106 | 108 | Linhares et al. (1984) |
| Adolescents | | | | |
| Finland | 60 | 63 | 34 | Lehotonen et al. (1999) |
| France | 49 | 71 | 21 | Guillemant et al. (2001) |
| Young adults | | | | |
| Norway | 70 | 81 | 53 | Vik et al. (1980) |
| Finland | 60 | | 46 | Lamberg-Allardt et al. (2001) |
| Germany | 51 | 70 | 30 | Zittermann et al. (1998) |
| Central and Western Europe | 45–55 | 68 | 42 | McKenna (1992)‖ |
| Turkey (women) | 39 | | | Alagol et al. (2000) |
| Group 1 | | 56† | | |
| Group 2 | | 32‡ | | |
| Group 3 | | 9§ | | |
| Elderly subjects | | | | |
| Norway | 61 | – | 47 | van der Wielen et al. (1995) |
| UK | 50–60 | 35 | 23 | Hegarty et al. (1994) |
| Italy | 42 | – | 28 | van der Wielen et al. (1995) |
| Greece | 35–38 | – | 24 | van der Wielen et al. (1995) |
| Institutionalized subjects | | | | |
| Switzerland | 47·5 | | 18 | Bischoff et al. (1999a) |
| France | 50 | | 8* | Fardellone et al. (1995) |

25(OH) D, 25-hydroxyvitamin D.
* Not differentiated by season.
† Dressed in a style which exposed the usual areas of the skin to sunlight.
‡ Traditional clothing with the skin of the hands and face uncovered.
§ Traditional Islamic style covering the whole body including hands and face.
‖ Mean level of different studies.

(Table 2). Moreover, dark-skinned Asian children living in England have low circulating 25(OH)D levels (Table 2) supporting the assumption that skin synthesis of vitamin D is critical for vitamin D status.

Generally, vitamin D status is more troublesome in elderly subjects in comparison with young adults (Table 2). Reasons for the low vitamin D status of elderly subjects are their often modest outdoor activities and the marked decrease in the capacity of human skin to produce vitamin D in elderly subjects in comparison with younger adults (Holick et al. 1989). The vitamin D status of elderly Norwegians is, however, more favourable in comparison with elderly subjects from other parts of Europe (Table 2), probably due to a higher oral vitamin D intake (see p. 564). The prevalence of serum 25(OH)D levels below 25 nmol/l is only 18 % in Norway and up to 83 % in Greece (van der Wielen et al. 1995). Nevertheless, Norwegians have mean 25(OH)D levels clearly below 100 nmol/l in winter and in summer. A very low vitamin D status is frequently observed in institutionalized elderly subjects (Table 2).

## Associations of low vitamin D status with chronic diseases

The following sections describe associations between low vitamin D status and various chronic diseases. At first, pathogenesis of the diseases is briefly explained. Then, epidemiological evidence for the vitamin D hypothesis is presented and available clinical intervention trials are critically reviewed.

### Osteopathy

Severe vitamin D deficiency results in an under-mineralization of the growing skeleton and in demineralization of the adult skeleton leading to rickets and osteomalacia, respectively. This is due to the marked suppression in intestinal Ca absorption and the impairment of Ca balance. There is now general agreement that an insufficient vitamin D status contributes to osteoporosis of the elderly. Low 25(OH)D levels are associated with low Ca absorption rates, hyperparathyroidism and increased bone turnover leading to bone loss (Ooms et al. 1995; Peacock, 1995). In elderly subjects, low circulating 25(OH)D levels are associated with a reduced bone mineral density at the proximal femur (Ooms et al. 1995; Scharla et al. 1996). It should also be mentioned that low bone mineral density due to an insufficient vitamin D status can reflect some stage of osteomalacia. The densitometric measurements only measure bone mineral content and density, which are low in osteomalacia and osteoporosis.

Even the transient decrease in vitamin D status during the lack of u.v. B irradiation in wintertime can lead to a transient loss of spinal bone mineral density in female subjects (Dawson-Hughes et al. 1991). Recent studies have also demonstrated that even in female adolescents insufficient 25(OH)D levels are associated with low forearm bone mineral density (Outila et al. 2001).

Several risk factors for hip fractures are at least in part related to a low vitamin D status. Incidence of hip fractures rises ten-fold in white women between the age of 75 and 95. The highest hip fracture rates are found in Northern Europe. Moreover, there is seasonality in the rates of hip fractures in the white US population with high rates in winter and low rates in summer in both sexes (Peacock, 1995). Patients with hip fractures more often have serum 25(OH)D levels below 50 nmol/l than control subjects of this age group (Diamond et al. 1998; LeBoff et al. 1999).

The results of controlled clinical trials with vitamin D administration on bone mineral density are inconsistent (Table 3). An increase in bone mineral density was observed in studies with vitamin D supplementation of 10·0, 17·5, and 375 μg/week. In studies with 20 μg vitamin D/d and 15 μg 25(OH)D/d no improvements were observed. In two out of these latter three studies mean dietary Ca intake was relatively low (530 and 570–740 mg/d) (Hunter et al. 2000; Peacock et al. 2000). It may well be that the amount of absorbed Ca was still too low to improve bone mineral density. Bone mineral loss at the femoral neck, spine, and total body could be prevented with a combined daily supplement of 17·5 μg vitamin D and 500 mg Ca (Dawson-Hughes et al. 1997). Moreover, a long-term randomized large controlled trial has shown that combined supplements of vitamin D (20 μg/d) and Ca (1 200 mg/d) were capable of preventing non-vertebral fractures in healthy ambulatory subjects (Chapuy et al. 1992). The bone density of the proximal femur increased 2·7 % in the vitamin $D_3$–Ca group and decreased 4·6 % in the placebo group. It seems probable that the anti-fracture effect of Ca and vitamin D supplementation is not only due to their effect on bone mineral density. An increase in 25(OH)D levels may improve neuromuscular coordination, as measured by body sway, and may thus decrease the risk of falling and falling-related fractures (see also p. 555).

### Myopathy

It has been assumed already at the beginning of the 20th century that severe vitamin D deficiency results in a disturbed muscle metabolism (Ritz et al. 1980). Animal studies have demonstrated that the aktinomyosin content of myofibrills is reduced during experimental rickets (Stroder & Arensmeyer, 1965). Moreover, vitamin D deficiency can impair intracellular Ca metabolism in muscle cells. The Ca content of mitochondria isolated from vitamin D-depleted chicks is low (Pleasure et al. 1979) and Ca uptake into the sarcoplasmic reticulum is reduced during vitamin D deficiency (Curry et al. 1983). Patients with osteomalacia suffer from muscle weakness and have low serum levels of muscle enzymes (Ritz et al. 1980; Rimaniol et al. 1994). Supplementation with 357 or 1250 μg vitamin D/d or 50 μg 25(OH)D/d for 1 to 2 months was able to normalize muscle strength in patients with myopathy (Rimaniol et al. 1994; Ziambaras & Dagogo-Jack, 1997). Sub-clinical myopathy may even occur at serum 25(OH)D levels of 10–50 nmol/l (Peacock, 1995). In line with this assumption, leg extension power was positively correlated with serum 25(OH)D levels in elderly males and with serum calcitriol levels in the

**Table 3.** Intervention trials with vitamin D or 25-hydroxyvitamin D (25(OH)D) on bone mineral density (BMD) and fracture risk in post-menopausal women and elderly men

| Reference | Study design | n | Duration of treatment (years) | Age (years) | Initial serum levels 25(OH)D (nmol/l) | Initial serum levels Calcitriol (pmol/l) | Treatment | Change in serum levels 25(OH)D (nmol/l) | Change in serum levels Calcitriol (pmol/l) | Results |
|---|---|---|---|---|---|---|---|---|---|---|
| Nordin et al. (1985) | PC | 137 F | 2 | 65–74 | 62 | n.d. | 375 µg Vitamin D$_2$/week | +68 | n.d. | Metacarpal cortical bone loss ↓ |
| Dawson-Hughes et al. (1991) | DBPC | 249 F | 1 | Mean 62 | 97* | 74* | 10 µg Vitamin D/d† | −5‡ | 0‡ | 1 % Less bone loss during wintertime v. placebo |
| Ooms et al. (1995) | DBPC | 48 F | 2 | Mean 80 | 27 | 111 | 10 µg Vitamin D/d | +35 | +4 | Change in BMD at femoral neck: +1·9 to +2·6 % v. placebo |
| Dawson-Hughes et al. (1995) | DBPC | 261 F | 2 | Mean 63 | n.d. | n.d. | 2·5 and 17·5 µg Vitamin D/d | n.d. | n.d. | Change in BMD at femoral neck: +1·5 % with 17·5 µg vitamin D v. 2·5 µg vitamin D |
| Hunter et al. (2000) | DBPC | 158 F | 2 | 47–70 | 71 | n.d. | 20 µg Vitamin D/d | +37 | n.d. | No effects on BMD v. controls |
| Patel et al. (2001) | DBPC | 70 F | 2 | 24–70 | 68 | n.d. | 20 µg Vitamin D/d | +25 | n.d. | No effects on BMD v. controls |
| Peacock et al. (2000) | DBPC | 438 F and M | 4 | 75 | 60·5 | 103 | 15 µg 25(OH)D/d | +58 | −15 to −25 | No effects on BMD v. controls |
| Graafmans et al. (1996) | PC | 81 F | 2 | 81 | 27 | 115 | 10 µg Vitamin D/d | +30 | +1 | Change in BMD +4·4 % and +4·2 % in the BB and Bb genotype v. placebo |

DBPC, double-blind, placebo-controlled; PC, plaebo-controlled; F, female, M, male; n.d., no data available; ↓, down.
* Summer values.
† Placebo and serum group were given 377 mg Ca/d.
‡ Winter values of the supplemented group v. summer values.

whole group of males and females. The males had mean 25(OH)D levels of 90 (SD 87·5) nmol/l and the females had mean 25(OH)D levels of 68 (SD 53) nmol/l (Bischoff *et al.* 1999*b*) indicating that a large number of subjects had an insufficient vitamin D status. A recent study has brought forward evidence that a low vitamin D status also contributes to the pathogenesis of congestive heart failure, a disease resulting in cardiac muscle weakness due to impaired myocardial contractility. Circulating levels of NT-proANP, a biochemical indicator of congestive heart failure severity, were inversely correlated with serum 25(OH)D levels ($r^2$ 0·16, $P < 0·001$; Zittermann *et al.* 2003).

Supplemental studies have demonstrated that doses of 0·5 μg calitriol/d or 10 μg vitamin D/d had no effects on parameters of muscle function (Table 4). A daily supplement of very high doses of vitamin D and also doses of 20 μg vitamin D/d could, however, significantly improve muscle function in subjects with low initial 25(OH)D levels (Table 4). It should also be mentioned that in both intervention trials the 20 μg vitamin D/d was combined with a daily supplement of 1 200 mg Ca. Probably, the combined effect of 20 μg vitamin D with high doses of oral Ca was responsible for the beneficial effects in these studies.

### Infections

There is mounting evidence for a pivotal role of vitamin D in the immune system. Monocytes, the leucocytes with the highest phagocytosis capacity, continuously exprime the vitamin D receptor (Bhalla *et al.* 1983). Calcitriol is able to induce the differentiation of monocytes into macrophages (Provvedini *et al.* 1986). Macrophages represent the first unspecific defence line of the immune system. Calcitriol increases the activity of lysosomal enzymes in macrophages and facilitate cytotoxic activity by enhancing the rate of phagocytosis. This latter effect is mediated by an enhanced expression of specific Fc-surface receptors (Boltz-Nitulescu *et al.* 1995) and by an increased respiratory burst (Cohen *et al.* 1986). Macrophages possess the enzyme 1α-hydroxylase and are, thus, able to produce calcitriol from 25(OH)D (Rigby, 1988). Activity of this enzyme is enhanced in activated macrophages leading to a marked increase of the local calcitriol concentration (Pryke *et al.* 1990).

Data relating infectious diseases to vitamin D status are scanty. However, there is some evidence from epidemiological data for a link between low vitamin D status and an increased risk for infections. The prevalence of acute respiratory infections was 81 % in Egyptian infants with nutritional rickets in comparison with 58 % in the control group (Lawson *et al.* 1987). In 500 Ethiopian children with pneumonia the incidence of rickets was thirteen times higher compared with 500 healthy children indicating that severe vitamin D deficiency was frequent in the patients with pneumonia (Muhe *et al.* 1997). Moreover, Rehman (1994) has published in a letter the results of a supplementation study with 150 μg vitamin D/week and 650 mg Ca/d in children who had previously repeatedly suffered from respiratory diseases. Treatment was performed for 6 weeks

and resulted in the absence of infectious disease for the following 6 months. Therapy also normalized the enhanced alkaline phosphatase and increased Ca serum levels, indicating that a sub-clinical vitamin D deficiency was responsible for the frequent infections.

Vitamin D status also seems to be involved in the risk of tuberculosis (Chan, 2000). *Mycobacterium tuberculosis* is an intracellular pathogen that resides predominantly within the macrophage. Reduced monocyte-macrophage function plays an important role in the pathogenesis of tuberculosis (Davies, 1985). Cross-sectional studies have indicated that patients with tuberculosis have lower 25(OH)D levels in comparison with control subjects (Davies *et al.* 1985, 1988; Chan *et al.* 1994). Serum 25(OH)D levels of the tuberculosis patients and the controls were 16 and 27 nmol/l, 46 and 69 nmol/l, and 52 and 95 nmol/l, respectively. Moreover, the prevalence of tuberculosis is enhanced in nursing-home residents (Woo *et al.* 1996). In the UK the incidence is high in Asian immigrants and especially in those immigrants living in the UK for only a short time. Obviously, Asians are infected in their country of origin, where the infection does not lead to overt disease due to plentiful sunlight and sufficient skin vitamin D synthesis. Migration towards a more northern latitude then results in an impaired vitamin D status. The outbreaks of tuberculosis usually occur within the first 5 years after arrival. The requirement of an infection with *M. tuberculosis* can also explain the low incidence of tuberculosis among Asians born in the UK (Chan, 2000). It has recently been demonstrated that the VDR genotype at the Taq1 restriction site influences susceptibility to tuberculosis indicating a role of vitamin D in the pathogenesis of the disease (Wilkinson *et al.* 2000).

### Inflammatory and autoimmune diseases

Experimental studies have demonstrated that calcitriol has a modulating effect on the specific immune system. Briefly, macrophage-derived cytokines induce resting T-helper (Th) cells to differentiate into Th0 cells. Under the influence of additional factors such as exogenous cytokines and co-stimulatory molecules expressed by antigen-presenting cells, these Th0 cells further differentiate into Th1 or into Th2 cells. Both T-cell subsets secrete a specific cytokine profile. These cytokines are involved in the proliferation and differentiation of T- and B-cells (Lemire *et al.* 1985; Provvedini *et al.* 1989; Müller *et al.* 1991*b*). Calcitriol can inhibit the synthesis of mRNA of the macrophages-derived cytokines interleukin (IL)-1, IL-6, IL-12 and tumour-necrosis factor α (TNF-α) (Müller *et al.* 1991*a*; D'Ambrosio *et al.* 1998). Moreover, calcitriol can decrease the antigen-presenting activity of macrophages to lymphocytes by a reduction of the expression of MHC-II molecules on the cell surface (Rigby *et al.* 1990). Calcitriol can also suppress the IL-2 secretion of Th1 cells (Lemire *et al.* 1995).

*Rheumatoid arthritis.* Rheumatoid arthritis is characterized by the infiltration of T lymphocytes, macrophages and plasma cells into the synovium, and the initiation of a chronic inflammatory state that involves overproduction of pro-inflammatory cytokines such as TNF-α and

IL-6 and a dysregulated Th1-type response. Rheumatoid arthritis patients have elevated levels of C-reactive protein, a biochemical indicator of inflammation. Epidemiological data indicate that more than 60 % of rheumatic patients have 25(OH)D levels below 50 nmol/l (Aguado et al. 2000) and that 16 % have levels in the range of vitamin D deficiency (<12·5 nmol/l; Kröger et al. 1993). In the general population, the risk for progression of osteoarthritis is already enhanced at a serum 25(OH)D level below 85 nmol/l and a vitamin D intake below 9·7 μg/d (McAlindon et al. 1996). Serum calcitriol levels are reduced in patients with a high disease activity compared with a low actual disease activity (Oelzner et al. 1998). Calcitriol is able to markedly suppress disease activity in an animal model of rheumatoid arthritis (DeLuca & Cantorna, 2001). Intervention trials with 1 μg 1α-vitamin D/d could, however, not demonstrate a significant effect on disease outcome in rheumatoid arthritis patients. In contrast, administration of 2 μg 1α-vitamin D/d and also the treatment with relatively high doses of vitamin D and 25(OH)D were able to significant improve pain symptomatology (Table 5).

While treatment with 1 μg 1α-vitamin D/d resulted only in a non-significant decrease in C-reactive protein, IL-6, and TNF-α levels (Hein & Oelzner, 2000), administration of 2 μg 1α-vitamin D/d was able to significantly reduce serum C-reactive protein levels (Andjelkovic et al. 1999). Unfortunately, C-reactive protein and cytokines were not measured in the earlier studies performed with 25(OH)D and vitamin D.

*Inflammatory bowel diseases.* Serum concentrations of 25(OH)D levels are low in patients with inflammatory bowel diseases such as ulcerative colitis and Crohn's disease (Jahnsen et al. 2002). Even newly diagnosed patients have lower 25(OH)D in comparison with controls (Lamb et al. 2002). Moreover, geographic variations of inflammatory bowel disease within the USA suggest that the amount of vitamin D available may be an important factor influencing disease development (Podolsky, 1991; Sonnenberg et al. 1991). The vitamin D hypothesis has been tested in an experimental model of IL-10 knockout mice (Cantorna et al. 2000). These animals spontaneously develop symptoms similar to those of human inflammatory bowel disease. The IL-10 knockout mice rapidly developed diarrhoea and cachexia and had a high mortality rate when they were made vitamin D-deficient. In contrast, vitamin D-sufficient IL-10 knockout mice did not develop diarrhoea, waste or die. Moreover, supplementation with vitamin D or calcitriol significantly ameliorated symptoms (Cantorna et al. 2000).

*Multiple sclerosis.* Multiple sclerosis (MS) is a demyellinating disease of the central nervous system that is debilitating and can be fatal (Hayes et al. 1997). Manifestation of the disease is typically between the years of 20 and 40. It appears that the pathological demyellinating of the central nervous system is caused by T-cell-mediated autoimmune processes. These alterations are obviously promoted by a genetic component and by virus infections and traumas. The prevalence of MS is nearly zero close to the equator and is markedly increased in regions of more northern latitudes (Dichgans & Diener, 1987). Moreover, there is a North to South gradient of the MS prevalence

in the USA (Schwartz, 1992). Exceptions from this general North to South gradient in the MS prevalence of the Northern hemisphere are some Swiss districts at high altitude (>2000 m), Greenland and the costal regions of Norway. In these regions a low MS prevalence was reported (Dichgans & Diener, 1987; Hayes et al. 1997). Results are consistent with the hypothesis that an inadequate vitamin D status is an important pathogenetic factor in MS. Annual u.v. B irradiation is more intensive in Swiss districts of high altitude than in regions of low altitudes. In Greenland and at the costal regions of Norway there is a traditionally high consumption of vitamin D-rich fatty fish (Hayes et al. 1997). A study set up to investigate bone health in MS patients revealed a prevalence of insufficient serum 25(OH)D levels (<50 nmol/l) in 77 % of the patients (Nieves et al. 1994). Experimental studies have shown that diets high in Ca and calcitriol can completely suppress the induction of autoimmune encephalomyelitis, which is a model of MS (Cantorna et al. 1996). Moreover, calcitriol can prevent the progression of autoimmune encephalomyelitis when Ca is high, but not when Ca is low in the diet (Cantorna et al. 1999). An intervention study in MS patients has demonstrated that daily supplementation with 16 mg Ca/kg body weight, 10 mg Mg/kg body weight and 125 μg vitamin D/d for 1–2 years was able to decrease the relapse rate of MS patients compared with the expected exacerbations (Goldberg et al. 1986). Several mechanisms have been held responsible for the beneficial effects of vitamin D in MS including an inhibition of inflammatory T-helper cells, an inhibition of the production of inflammatory cytokines by activated macrophages, an enhanced production of anti-inflammatory cytokines, and an anti-proliferative action in lymphocytes by the expression of VDR (Hayes et al. 1997). In line with these assumptions it has recently been demonstrated that vitamin D supplementation is able to reduce IL-2 mRNA in peripheral blood mononuclear cells of MS patients (Cantorna et al. 2001).

### Hypertension, cardiovascular diseases and diabetes mellitus

*Hypertension.* Essential hypertension is related to several disturbances in systemic and cellular Ca metabolism. Extracellular ionized or ultrafiltrable Ca levels are decreased while intracellular cytosolic Ca concentrations are increased (McCarron et al. 1987). Dietary Ca intake is often lower (McCarron et al. 1987) and renal Ca loss is higher in hypertensive than in normotensive subjects (Strazzullo, 1991; MacGregor & Cappuccio, 1993) indicating a renal Ca leak. Epidemiological studies have demonstrated a weak inverse association between serum 25(OH)D levels and diastolic blood pressure in population groups with mean 25(OH)D levels of 30–50 nmol/l (Scragg et al. 1992). Moreover, Afro-Americans have a significantly higher prevalence of diastolic hypertension (Dustan, 1990) and have lower 25(OH)D levels (Harris & Dawson-Hughes, 1998) compared with white Americans. In clinical trials, daily administration of 5 μg vitamin D showed no effects on blood pressure in normotensive subjects (Table 6). Some but not all studies have, however,

**Table 4.** Intervention studies with vitamin D or calcitriol on parameters of muscle function such as muscle strength, body sway, and/or falls

| Reference | Study design | Duration of treatment (months) | n | Mean age (years) | Initial serum levels | | Treatment | Change in serum levels | | Results |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 25(OH)D (nmol/l) | Calcitriol (pmol/l) | | 25(OH)D (nmol/l) | Calcitriol (pmol/l) | |
| Grady et al. (1991) | PC | 6 | 98 | 69 | 60 | 86 | 0·5 µg Calcitriol/d | n.d. | +0 | No improvement |
| Graafmans et al. (1996) | PC | 7 | 354 | >70 | n.d. | n.d. | 10 µg Vitamin D/d | n.d. | n.d. | No improvement |
| Glerup et al. (2000) | PC | 6 | 55 | 32 | 6·7 | 108 | 2800 µg Vitamin D/month | +28 | +15 | Improved maximal voluntary knee extension |
| Pfeifer et al. (2000) | DBPC | 2 | 148 | 74 | 26 | 91 | 20 µg Vitamin D/d* | +40 | +36 | Decrease in body sway and number of falls |
| Bischoff et al. (2001) | DBPC | 6 | 122 | 84 | 12 | n.d. | 20 µg Vitamin D/d | n.d. | n.d. | Improved functional measures and decrease in number of falls |

PC, Placebo-controlled; DBPC, double-blind, placebo-controlled; 25(OH)D, 25-hydroxyvitamin D; n.d., no data available.
* An additional Ca supplement of 1 200 mg/d was given to the placebo and the verum group.

**Table 5.** Intervention trials with vitamin D and its metabolites on disease activity in patients with rheumatoid arthritis

| Reference | Study design | Duration of treatment | n | Age (years) | Initial serum levels | | Treatment | Change in serum levels | | Results |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 25(OH) D (nmol/l) | Calcitriol (pmol/l) | | 25(OH)D (nmol/l) | Calcitriol (pmol/l) | |
| Hein & Oelzner (2000) | Open trial | 8 weeks | 20 | 26–78 | n.d. | 95 | 1 µg 1α-Vitamin D/d | n.d. | +5 | No effects |
| Yamauchi et al. (1989) | DBPC | 16 weeks | 140 | – | n.d. | n.d. | 1 or 2 µg 1α-Vitamin D/d | n.d. | n.d. | No effects |
| Andjelkovic et al. (1999) | Open trial | 3 months | 19 | 23–71 | n.d. | n.d. | 2 µg 1α-Vitamin D/d | n.d. | n.d. | Decreased disease activity |
| Brohult & Jonson (1973) | DBPC | 1–2 years | 49 | 18–69 | n.d. | n.d. | 2500 µg Vitamin D/d | n.d. | n.d. | Decreased disease activity |
| Dottori et al. (1982) | PC | 30 d | 45 | 20–64 | n.d. | n.d. | 50 µg 25(OH)D/d | n.d. | n.d. | Improved pain symtomatology |

DBPC, double-blind, placebo-controlled; PC, placebo-controlled; 25(OH)D, 25-hydroxyvitamin D; n.d., no data available.

demonstrated a blood pressure-lowering effect with 0·75 or 1·0 μg 1α-vitamin D/d in hypertensive patients (Table 6). Short-term supplementation with 20 μg vitamin D/d (in combination with a supplement of 1 200 mg Ca/d) was able to significantly reduce diastolic blood pressure. A reduction in diastolic and systolic blood pressure was observed in mildly hypertensive patients after 6 weeks of u.v. B exposure but not after u.v. A exposure (Table 6). A normalization of the enhanced intracellular Ca levels seems to be an important measure in order to reduce blood pressure. This can explain the therapeutic effects of Ca channel blockers in hypertensive patients (McCarron et al. 1987). A low adenylate cyclase activity can result in a decreased Ca re-uptake into the sarcoplasmic reticulum (Curry et al. 1974) and can contribute to an accumulation of intracellular free Ca, and to an increase in vascular reactivity and blood pressure (McCarron et al. 1987). Activity of the intracellular adenylate cyclase is calcitriol-dependent (Nemere et al. 1993) and improvement of the activity of this enzyme may thus reduce free cellular Ca concentrations.

*Cardiovascular diseases.* Dyslipoproteinaemia, disturbed glucose tolerance, and an increase in blood coagulation factors, blood viscosity, and leucocyte counts are important risk factors for the development of arteriosclerosis (Mendall et al. 1997). There is now increasing evidence that arteriosclerosis is a low-grade systemic inflammatory disease. An increase in serum C-reactive protein levels is an important indicator of inflammatory reactions and also of the risk of developing arteriosclerosis (van Lente, 2000). Synthesis of C-reactive protein is regulated by IL-6 and TNF-α (Mendall et al. 1997). Animal studies have demonstrated that IL-6 accelerates arteriosclerosis (Huber et al. 1999). Calcitriol can suppress the secretion of TNF-α and IL-6 in vitro in a dose-dependent manner (Müller et al. 1992). We have recently observed an inverse association between TNF-α and 25(OH)D levels in human subjects (r 0·30, P<0·01; Zittermann et al. 2003). Epidemiological investigations brought forward evidence for an inverse association between myocardial infarction and plasma 25-hydroxyvitamin $D_3$ levels (Scragg et al. 1990). Moreover, the nadir of 25(OH)D levels in the UK during wintertime (Hegarty et al. 1994) is paralleled by an increased cardiovascular morbidity (Douglas et al. 1991). Since the prevalence of cardiovascular diseases is low in alpine regions of high altitudes and low temperatures (Scragg, 1981), reasons apart from ambient temperature must be responsible for the differences in cardiovascular diseases between different seasons. One factor may be the low vitamin D availability in winter, while vitamin D availability is high in alpine regions due to intensive u.v. B exposure. It is also only an apparent paradox that Eskimos have a low risk of arteriosclerosis (Feskens & Kromhout, 1993) although u.v. B irradiation is low in the region they live. The traditional diet of Eskimos is high in marine fishes and other sea meat (Feskens & Kromhout, 1993). These foods are very rich in vitamin D (Table 7).

Physical activity and an increased intake of unsaturated fatty acids are frequently recommended in the prevention and therapy of cardiovascular diseases. Physical activity is associated with higher circulating levels of 25(OH)D and calcitriol compared with a sedentary lifestyle (Zittermann et al. 2000). Consequently, the beneficial effect of physical activity may at least in part be explained by the improvement in vitamin D status. Physiological amounts of unsaturated fatty acids can reduce the binding of serum calcitriol to the vitamin D-binding protein by more than 20 % and can thus increase the bioavailability of calcitriol. Saturated fatty acids do not show such an effect (Bouillon et al. 1992).

*Diabetes mellitus.* The dependence of normal insulin secretion in pancreatic β-cells on vitamin D has been known for several decades. Experimental studies have demonstrated that a reduction in vitamin D activity can result in both increased insulin resistance and reduced insulin secretion (Boucher, 1998). Epidemiological data have shown a four- to five-fold higher prevalence of non-insulin-dependent diabetes in dark-skinned Asian immigrants in comparison with British Caucasians indicating that low vitamin D status may contribute to the pathogenesis of diabetes (McKeigue et al. 1992). Moreover, in elderly subjects the subgroup with the lowest tertile of 25(OH)D levels had a significantly higher blood glucose increase and higher blood insulin increase after an oral glucose-tolerance test in comparison with the subgroup with the highest tertile of 25(OH)D levels (Baynes et al. 1997). Data indicate that vitamin D insufficiency may result in insulin resistance. Results are in line with the suggestion that enhanced levels of TNF-α, a cytokine with is inversely related to 25(OH)D and calcitriol (see p. 560), promote insulin resistance (Hotamisligil & Spiegelman, 1994).

A severe vitamin D deficiency probably results in low serum insulin levels indicating reduced insulin secretion (Boucher, 1998). In uraemic patients, administration of 1α-vitamin D was able to improve blood glucose levels and increase serum insulin levels (Table 8). Moreover, two studies have demonstrated that daily administration of 50 μg and 1050–2125 μg vitamin D/d was able to reduce blood glucose levels in patients with osteomalacia. In another study, however, there was an increase in blood glucose levels in diabetic patients 8–12 weeks after a single injection of 2500 μg vitamin D compared with the pre-treatment value (Table 8). It was assumed by the authors of that study that the failure to correct diabetes was probably due to the modest increase of 25(OH)D levels of only 25 nmol/l (Boucher et al. 1995).

A Norwegian study brought forward evidence that the daily intake of cod-liver oil during pregnancy can reduce the risk of diabetes in the offspring (Stene et al. 2000). Cod-liver oil has a very high vitamin D content (Table 7). In a more recent Finnish investigation, regular vitamin D supplementation of 50 μg/d during infancy in the 1960s was associated with a markedly reduction in the risk of type 1 diabetes 30 years later in comparison with unsupplemented infants (relative risk 0·12). Children suspected of having rickets during the first year of life had a threefold increased prevalence of type 1 diabetes in comparison with those without such a suspicion (Hyppönen et al. 2001). In Germany, the incidence of type 1 diabetes in adolescents is higher in autumn and winter compared with spring and summer (Statistisches Bundesamt, 1998). Autoimmune processes are regarded to play an important

**Table 6.** Intervention trials with u.v.B irradiation and supplementation with vitamin D and its metabolites on blood pressure in normotensive and hypertensive patients

| Reference | Study design | n | Duration of treatment | Age (years) | Initial serum levels | | Treatment | Change in serum levels | | Results |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 25(OH)D (nmol/l) | Calcitriol (pmol/l) | | 25(OH)D (nmol/l) | Calcitriol (pmol/l) | |
| Lind et al. (1987) | DBPC | 26 H | 6 months | Mean 63 | n.d. | n.d. | 1 μg 1 α-Vitamin D/d | n.d. | n.d. | −9·2 mmHg diastolic blood pressure |
| Lind et al. (1988) | DBPC | 65 H | 12 weeks | 61−65 | n.d. | n.d. | 0·75 μg 1α-Vitamin D/d | n.d. | n.d. | −9/ − 3 mmHg systolic and diastolic blood pressure |
| Lind et al. (1989) | DBPC | 39 H | 4 months | Mean 51 | n.d. | n.d. | 1 μg 1α-Vitamin D/d | n.d. | n.d. | No difference v. controls |
| Pan et al. (1993) | DBPC | 58 N | 11 weeks | 63−83 | 61 | n.d. | 5 μg Vitamin D₃ | +7 | n.d. | No effects |
| | | | | | 59 | n.d. | 5 μg D₃+800 mg Ca/d | +11 | n.d. | |
| Scragg et al. (1995) | DBPC | 189 N | 5 weeks | 63−76 | 34·5 | n.d. | 5 μg Vitamin D/d | +14 | n.d. | No effects |
| Krause et al. (1998) | DBPC | 18 H | 6 weeks | 26−66 | 58 | n.d. | u.v.B irradiation thrice-weekly | +94 | n.d. | −6/ − 6 mmHg systolic and diastolic blood pressure |
| Pfeifer et al. (2001) | DBPC | Elderly women | 8 weeks | 75 | 26 | 91 | 20 μg Vitamin D/d* | +40 | +36 | −6 mmHg systolic |

DBPC, double-blind, placebo-controlled; H, hypertensive subjects (blood pressure ≧140/90); N, normotensive subjects; 25(OH)D, 25-hydroxyvitamin D; n.d., no data available.
* An additional Ca supplement of 1 200 mg/d was given to the placebo and the verum group.

**Table 7.** Adequate daily vitamin D intake values by age group for Germany, Austria, and Switzerland, and vitamin D content of some foods per 100 g edible portion (from Souci *et al.* 1994; Deutsche Gesellschaft für Ernährung *et al.* 2000)

| Age group | Vitamin D adequate intake ($\mu$g) | Food | Vitamin D contact ($\mu$g) |
|---|---|---|---|
| Infants | 10 | Herring | 27 |
| Children | 5 | Eel | 20 |
| Adolescents | 5 | Salmon | 16 |
| Adults < 65 years | 5 | Cod | 1·3 |
| Adults > 65 years | 10 | Butter | 1·3 |
| | | Egg | 3 |
| | | Pork liver | – |
| | | Cod-liver oil | 330 |

role in the pathogenesis of type 1 diabetes. Again, it should be mentioned that calcitriol has immunomodulatory properties (see p. 557). Availability of calcitriol in the cell may thus influence autoimmune processes. The vitamin D hypothesis is also in line with results demonstrating that the risk of type 1 diabetes and of type 2 diabetes is influenced by the VDR genotype at the BmsI restriction site (Chang *et al.* 2000; Ortlepp *et al.* 2001).

It should be mentioned that hypertension, cardiovascular diseases, and diabetes mellitus are often associated with obesity. Obese subjects have an increased risk for low circulating 25(OH)D levels (Bell *et al.* 1985; Wortsman *et al.* 2000) due to the storage of vitamin D and 25(OH)D in adipose tissue (Wortsman *et al.* 2000). The alterations in vitamin D metabolism of obese subjects in comparison with lean subjects are also associated with functional alterations such as elevated PTH levels (Bell *et al.* 1985; Wortsman *et al.* 2000). Obesity might thus contribute to insufficient circulating 25(OH)D levels.

### Cancer

Although carcinogenesis can occur relatively quickly, most cancers develop over decades making it difficult to perform reliable human intervention studies on the association between vitamin D and cancer risk. However, there is evidence that enhanced sunlight exposure is associated with lower prostate, breast and colon cancer death rates, while the historical geographical distribution of rickets parallels that for these cancer deaths (Guyton *et al.* 2001).

The strongest epidemiological evidence supporting a protective role for vitamin D in colon cancer is from prospective studies. Inverse associations for vitamin D intake and colon or colorectal cancer with relative risks ranging from 0·33 to 0·74 have been reported (Bostick *et al.* 1993; Guyton *et al.* 2001). Moreover, a nested case–control study based on serum drawn from a cohort of 25 620 individuals reported that concentrations of 25(OH)D in the range of 65–100 nmol/l were associated with large reductions in the incidence of colorectal cancer compared with lower 25(OH)D levels (Garland *et al.* 1991).

A reduced risk of breast cancer has been observed in the NHANES I epidemiological follow-up study with several measures of sunlight exposure and dietary vitamin D intake, with relative risks ranging from 0·67 to 0·85. The risk reductions were highest for women who lived in US regions of high solar radiation and no reduction was founds for women who lived in regions of low solar radiation (John *et al.* 1999). Data are in line with experimental results suggesting that high amounts of vitamin D and dietary Ca decrease susceptibility to chemically induced mammary neoplasia (Carroll *et al.* 1991).

Another important observation is that in the USA the occurrence of prostate cancer and MS have similar geographical distributions (Schwartz, 1992). The hypothesis of a vitamin D dependency on prostate cancer has recently been confirmed by a large nested case–control study (Tuohimaa *et al.* 2001). In a 13-year follow-up study of about 19 000 middle-aged Finnish men, prostate cancer risk was highest among the group of younger men (40–51 years) with low serum 25(OH)D levels. Approximately one half of the serum samples had 25(OH)D levels below 50 nmol/l. Low serum 25(OH)D levels, however, appeared not to increase the risk of prostate cancer in older men (>51 years). Data suggest that vitamin D has a protective role against prostate cancer only before the andropause, when serum androgen concentrations are higher. The lowest 25(OH)D concentrations in the younger men were associated with more aggressive prostate cancer (Tuohimaa *et al.* 2001).

Vitamin D is anti-proliferative and promotes cellular maturation, induces differentiation and apoptosis in many different cell lines including malignant cells (Feldman *et al.* 1995; Guyton *et al.* 2001). Vitamin D receptors have been found in the mammary gland, in the colon and in the prostate (Table 1). Moreover, it is now recognized that colon, breast, and prostate cells also express the 1$\alpha$-hydroxylase to form calcitriol from circulating 25(OH)D (Holick, 2002). It seems clear that vitamin D must be viewed as an important cellular anti-tumour substance.

### Prevention of vitamin D insufficiency

Preventive measures have to consider that there is a high risk of vitamin D insufficiency in the whole population during winter and that the elderly population, and especially institutionalized subjects, are at increased risk for vitamin D insufficiency or even deficiency. Available modes of prevention are threefold: increased exposure to

**Table 8.** Intervention trials with vitamin D and its metabolites on blood glucose and insulin levels in patients with diabetes mellitus and/or disturbed calcium and vitamin D metabolism

| Reference | Study design | n | Duration of treatment | Age (years) | Initial serum levels 25(OH)D (nmol/l) | Calcitriol (pmol/l) | Treatment | Change in serum levels 25(OH)D (nmol/l) | Calcitriol (pmol/l) | Results |
|---|---|---|---|---|---|---|---|---|---|---|
| Rudnicki & Molsted-Pedersen (1997) | Open trial | 20 GD | 2 h | n.d | n.d. | 240 | Intravenous infusion of 2 μg calcitriol/m² | n.d. | +115 | Blood glucose: 0·8 mmol/l ↓ after GTT |
| Ljunghall et al. (1987) | PC | 65 IP | 3 months | 61–65 | 93 | 110 | 0·75 μg 1α-Vitamin D/d | +12 | +5 | No improvement |
| Boucher et al. (1995) | Open trial | 22 DP | 8–12 weeks | n.d. | 9 | n.d. | Single intramuscular injection of 2500 μg Vitamin D | +25 | n.d. | Blood glucose,1·8 mmol/l ↑; specific insulin, 43 mU/l ↑ after GTT |
| Kocian (1992) | Open trial | 61 OP | 6 weeks | n.d. | n.d. | n.d. | 1050–2125 μg Vitamin D/d and 470 700 mg Ca/d | n.d. | n.d. | Blood glucose, 1·5 mmol/l ↓ after GTT |
| Kumar et al. (1994) | Case report | 1 HP | 5 months | 65 | 6·8 | 30 | 50 μg Vitamin D/d | +46 | +88 | Blood glucose, 1·6 mmol/l ↓; insulin, 29 mU/l ↑ after GTT |
| Türk et al. (1992) | PC | 31 UP | 8 weeks | 17–66 | n.d. | 44 | 0·5 μg Calcitriol/d | n.d. | +103 | Blood glucose, 1·1 mmol/l ↓; insulin, 78 μIU/l ↑ after GTT |
| Allegra et al. (1994) | Open trial | 17 UP | 3 weeks | Mean 50 | n.d. | 44 | 0·5 μg Calcitriol/d | n.d. | +37 | Blood glucose, 1·1 mmol/l ↓; insulin, 13μ IU/l ↑ after GTT |

PC, placebo-controlled; GD, patients with gestational diabetes; IP, patients with impaired glucose tolerance; DP, vitamin D-deficient patients with impaired glucose tolerance; OP, patients with osteomalacia; HP, hypo-calcaemic patient; UP uraemic patients; 25(OH)D, 25-hydroxyvitamin D; GTT, glucose tolerance test; n.d., no data available; ↓, down; ↑, up.

u.v. light; dermal vitamin D application; increased oral vitamin D intake.

A general recommendation from health authorities of a higher sunlight exposure of the free-living population during winter may be largely ineffective due to the lack of u.v. B irradiation. In addition, there are valid concerns about photo-ageing and skin cancer if u.v. B exposure is increased, for example, between spring and autumn (McKenna, 1992). The vitamin D status of elderly people may be enhanced by skin exposure to artificial u.v. light, but provision of fluorescent lighting in wards has resulted in inconsistent responses and can be associated with complications; namely skin burns, keratoconjunctivitis, and cataracts (McKenna, 1992).

Similar to the administration of oestrogens, dermal application of vitamin D might be a useful individual measure to achieve constant levels of this steroid substance during several weeks. However, such a measure has not yet been tested in clinical trials.

Adequate daily oral vitamin D intake could be an easy and effective measure to maintain a physiological vitamin D status. Adequate intake values are 5–10 μg/d (National Nutrition Council, 1999; Deutsche Gesellschaft für Ernährung et al. 2000; Health Council of the Netherlands, 2000) and 15 μg vitamin D/d for elderly subjects with insufficient skin vitamin D synthesis (Health Council of the Netherlands, 2000). In Germany the mean vitamin D intake is 3 μg/d in females and 4 μg/d in males (Heseker et al. 1994). In The Netherlands vitamin D intakes are also below 5 μg/d (Health Council of the Netherlands, 2000). In middle-aged Finnish females and males mean vitamin D intake is 4·7 and 5·6 μg/d respectively (Lamberg-Allardt et al. 2001). Norwegians have a high consumption of vitamin D-rich fatty fish (Hayes et al. 1997) and usually consume cod-liver oil during their whole lifespan. This may explain the 'relatively' high 25(OH)D levels in elderly Norwegians during wintertime (Table 2).

The low vitamin D intake in several European countries is due to the fact that only a few foods are naturally good sources of vitamin D and some fishes alone can substantially contribute to an adequate nutritional supply of vitamin D (Table 7). Moreover, only a few foods are fortified with low amounts of vitamin D in Europe; for example, margarine, vegetable oil, cereals, breakfast beverages, and breads (Lips et al. 1996). The European Union is therefore supporting a project towards a strategy for optimal vitamin D fortification named OPTIFORD (Andersen et al. 2001).

It must be emphasized that currently no recommended intake level for vitamin D exists. The adequate intake values are crude estimates in order to prevent vitamin D-dependent diseases such as rickets and osteomalacia. We are probably ignoring the evidence that a much higher oral vitamin D intake than 5–15 μg/d is necessary to maintain adequate circulating 25(OH)D levels in the absence of u.v. B irradiation of the skin. Dose–response studies with daily doses of oral vitamin D have demonstrated that 10, 25, 100 and 250 μg vitamin D result in a mean increase of circulating 25(OH)D levels of 45, 48, 56, and 112 nmol/l, respectively (Vieth, 1999; Vieth et al. 2001a). Data from Tables 3, 4, 6 and 8 indicate that

vitamin D intakes of 5, 10, 20 and 50 μg/d increase mean serum 25(OH)D levels by 7–14, 30–35, 25–40, and 46 nmol/l respectively. Results suggest that the increase of 25(OH)D following a vitamin D supplement of 10 μg is often too low to maintain a 25(OH)D level above 50 nmol/l or even above 100 nmol/l. In line with this assumption mean circulating 25(OH)D levels were only 17·5 nmol/l in veiled ethnic Danish Moslem women although their estimated daily vitamin D intake was 13·5 μg/d (Glerup et al. 2000). Moreover, in Finnish adolescent girls daily supplementation with 10 μg Vitamin D₂ was not able to increase serum 25(OH)D levels during the winter. Supplementation with 20 μg vitamin D/d resulted in a 25(OH)D level which was only 14 nmol/l higher in comparison with the unsupplemented group (Lehtonen-Veromaa et al. 2002). In Danish perimenopausal women who took vitamin D supplements at least during wintertime, serum 25(OH)D levels were only 8·5 nmol/l higher than in non-users (Brot et al. 2001). Together, these data indicate that in the absence of u.v. B exposure the oral vitamin D demand is probably far above the present recommendation of 10 μg/d and may be up to 100 μg/d in order to maintain adequate circulating 25(OH)D levels (Heaney, 2000). Only those traditional diets with a regular intake of cod-liver oil and/or sea foods such as salmon, herring, and eel are able to provide such high amounts of vitamin D daily (Table 7). This also means that intervention trials with oral vitamin D supplements must obviously include much higher doses than the currently often-used 5–20 μg/d. Due to the relatively small increase in serum 25(OH)D levels and relatively low Ca absorption rates, vitamin D intakes of 5–20 μg/d alone may be inadequate to significantly improve the amount of absorbed Ca. A simple increase in oral Ca just as well as a high vitamin D intake can increase the amount of absorbed Ca. This may explain why some improvements in fracture prevalence, muscle function and blood pressure have been achieved with daily supplements of 20 μg vitamin D in combination with 1200 mg Ca/d (Chapuy et al. 1992; Pfeifer et al. 2000, 2001). Nevertheless, it is doubtful whether high oral Ca intake alone can beneficially influence intracellular Ca and cytokine metabolism. It is therefore encouraging that oral doses of vitamin D or 25(OH)D ≧ 50 μg/d were able to improve the disease outcome in patients with rheumatoid arthritis (Dottori et al. 1982) and MS (Goldberg et al. 1986). Moreover, administration of 50 μg vitamin D/d to infants can obviously markedly reduce the prevalence of type 1 diabetes in later life (Hyppönen et al. 2001). Data are a further indication that currently used oral vitamin D doses are probably much too low in the prevention and therapy of vitamin D-related diseases.

## Vitamin D intoxication

There are no reports of vitamin D intoxication in healthy adults after intensive sunlight exposure. Vitamin D in the skin reaches a plateau after only 15–30 min of u.v. B exposure. Then, vitamin D-inactive substances such as lumisterol and tachysterol are produced, which do not reach the systemic circulation. Thus, the maximum

25(OH)D level corresponding to an intensive u.v. B exposure can be regarded as an upper safe level (Fig. 2).

The changes in circulating calcitriol levels during intoxication are generally small (Markestad *et al.* 1987; Jacobus *et al.* 1992). Nevertheless, increases in serum calcitriol have been reported and might contribute to the symptoms of vitamin D intoxication such as hypercalcaemia (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board and Institute of Medicine, 1997). Hypercalcaemia results primarily from intestinal Ca hyperabsorption and to a lesser degree from Ca release from bone (Chesney, 1989). The hypercalcaemia induced by high oral doses of vitamin D can lead to nephrocalcinosis and coronary sclerosis (Hesse & Jahreis, 1990).

In all cases of vitamin D intoxication 25(OH)D levels were clearly above 200 nmol/l. Levels up to 1000 nmol/l and more have been observed (Markestad *et al.* 1987; Jacobus *et al.* 1992). All these instances of intoxication were the result of an excessive oral intake of vitamin $D_2$ or vitamin $D_3$. They are the result of an unregulated intestinal vitamin D uptake in association with an uncontrolled hepatic 25-hydroxylation leading to high circulating 25(OH)D levels. Vitamin D intoxication has been described in British infants during the late 1940s and early 1950s after heavy enrichment of dried milk powder together with vitamin D-enriched cereals and in addition to the recommendation of a daily vitamin D supplement of 17·5–20·0 μg (Chesney, 1989). Moreover, the 'stoss prophylaxis' in the former German Democratic Republic against rickets with intermittent doses as high as 15 mg vitamin $D_2$ was associated with symptoms of vitamin D intoxication such as hypercalcaemia and serum 25(OH)D levels of several hundred nmol/l (Markestad *et al.* 1987). In adults, vitamin D intoxication has been observed after the administration of very high therapeutic vitamin $D_3$ doses (Lilienfeld-Toal *et al.* 1978), in association with an over-the-counter supplement that contained 26 to 430 times the vitamin $D_3$ amount listed by the manufacturer (Koutka *et al.* 2001), and in association with an accidentally excessive overfortification of consumers' milk with vitamin $D_3$ (Jacobus *et al.* 1992; Blank *et al.* 1995). There are no reports in the literature about vitamin D intoxication with traditionally consumed foods (Chesney, 1989).

The US Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board and Institute of Medicine (1997) has defined a tolerable upper intake level of 25 μg vitamin D/d for infants and of 50 μg vitamin D/d for children aged >1 year and for adults (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board and Institute of Medicine, 1997). However, evidence for the threshold of 25 μg/d in infants is not well documented. Most of the occurrences of intoxication occurred during a time when circulating 25(OH)D could not be measured. Consequently, insufficient data of minimal vitamin D intake, corresponding serum 25(OH)D levels, and toxic effects are available in infants. Moreover, recent investigations suggest that an oral vitamin D intake up to 100 μg/d is safe in the adult population. No changes in serum and urinary Ca levels have been observed with

that dose. The highest individual 25(OH)D level after administration of 100 μg vitamin D/d was 140 nmol/l (Vieth *et al.* 2001*a*) and was, thus, in the range also seen during intensive u.v. B-exposure.

## Conclusions

Calcitriol is a very potent steroid hormone and is, on a molar basis, the most effective vitamin D metabolite. Nevertheless, an adequate serum 25(OH)D level is also necessary to achieve full physiological vitamin D activity. Obviously, the serum 25(OH)D level and not the serum calcitriol level is the best indicator for vitamin D insufficiency, adequacy, or toxicity.

Because only a few foods, especially some fatty fishes, naturally contain vitamin D in relevant amounts circulating 25(OH)D levels normally largely depend on u.v. B exposure. In tropical and subtropical regions, where more than 90 % of human evolution took place, u.v. B irradiation is abundant throughout the year. Reasons for a low vitamin D status in Europe are: (i) the seasonal lack of u.v. B irradiation; (ii) low outdoor activities; (iii) the ageing process leading to a reduced vitamin D synthesis in the skin; (iv) the low vitamin D content of most foods. Probably, the prevalence of a low vitamin D status will increase in future due to the rising number of elderly individuals in European societies, and due to the migration of dark-skinned people and veiled women to Europe. The relevance of the frequently low vitamin D status is not completely clear. However, there is growing evidence for the contribution of a circulating 25(OH)D level below 50 nmol/l to the development of various chronic diseases which are frequent in Western societies. Current estimations for an adequate oral intake are obviously much too low to achieve an optimal vitamin D status and thus to effectively prevent chronic vitamin D-dependent diseases.

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