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See page 25 for Upper Limits

Infants 400 IU
2-10 1,000 IU
11+ 2,000 IU

Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Vitamin D

(expressed on 4 December 2002)

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FOREWORD

This opinion is one in the series of opinions of the Scientific Committee on Food (SCF) on the upper levels of vitamins and minerals. The terms of reference given by the European Commission for this task, the related background and the guidelines used by the Committee to develop tolerable upper intake levels for vitamins and minerals used in this opinion, which were expressed by the SCF on 19 October 2000, are available on the Internet at the pages of the SCF, at the address: http://www.europa.eu.int/comm/food/fs/sc/scf/index_en.html.

1. INTRODUCTION

The principal physiological function of vitamin D in all vertebrates including humans is to maintain serum calcium and phosphorus concentrations in a range that support cellular processes, neuromuscular function, and bone ossification. Vitamin D accomplishes this goal by enhancing the efficiency of the small intestine to absorb dietary calcium and phosphorous, and by mobilising calcium and phosphorus from the bone (Holick, 1999; Holick *et al.*, 1998).

The last couple of decades it has become increasingly apparent that vitamin D also has other important functions in tissues not primarily related to mineral metabolism (Brown *et al.*, 1999; Holick, 1999). One example is the haematopoietic system, in which vitamin D affects cell differentiation and proliferation including such effects also in cancer cells. Vitamin D furthermore participates in the process of insulin secretion. The active metabolite of vitamin D, $1,25(OH)_2D$, regulate the transcription of a large number of genes through binding to a transcription factor, the vitamin D receptor (VDR).

Blood levels of vitamin D are influenced both by dietary intake and the amount of daylight exposure to the skin. Exposure of the skin to ultraviolet light catalyses the synthesis of vitamin D_3 (cholecalciferol) from 7-dehydrocholesterol. Thus vitamin D is more like a hormone and not strictly a vitamin according to the classical criteria that an essential nutrient is a substance the body cannot synthesise in sufficient quantities itself. Deprived of exposure to sunlight vitamin D becomes an essential nutrient. The effectiveness of exposure to sunlight or ultraviolet light in curing or preventing rickets was shown early in the twentieth century (Holick, 1995).

2. NUTRITIONAL BACKGROUND

2.1 Vitamin D supply

2.1.1 Vitamin D forms in food

Vitamin D comprises two closely related substances of nutritional importance: vitamin D_3 (cholecalciferol), which is the physiological form, and the synthetic analogue vitamin D_2

(ergocalciferol). The two forms only differ by the side chain to the sterol skeleton (Holick, 1999). It has been assumed, based on studies in the 1930s showing no conclusive difference between vitamin D_3 (from cod liver oil) and D_2 in their preventing effect against infantile rickets, that vitamin D_2 for practical purposes could be regarded as equal to vitamin D from cod liver oil. There is no contemporary evidence showing that vitamin D_3 and D_2 are equally efficient in increasing the circulating metabolite proximate to the active form. Indeed, later studies have shown important biological differences in this respect between these forms (Trang *et al.*, 1998). (See 2.5.2 for further details).

Vitamin D_3 and vitamin D_2, together with the provitamins they are made from, are all derivatives of sterols, their chemical structure resembles cholesterol, bile acids and the sex hormones. Vitamin D_2 is formed by UV radiation from its precursor ergosterol. Ergosterol is found in plants, especially yeast and fungi. The synthesis of ergocalciferol from ergosterol hardly takes place in nature. Plants are thus a poor source of vitamin D_2. Synthetic vitamin D_2 produced by irradiation of ergosterol used to be the form added to food or given as supplements. During the past two decades, vitamin D_3 has also been used to fortify milk, margarine and other foods worldwide, and although the use of vitamin D_2 in food and supplements still is widely used, its use is less than before. Vitamin D_3 is formed from its precursor 7-dehydrocholesterol, which is found in ample amounts in the skin and fat depots in animals and man. Vitamin D is relatively stable in fat solutions, e.g. is not inactivated by pasteurisation or sterilisation. It oxidises in contact with air and in acid solutions and is inactivated when exposed to sunlight.

2.1.2 Vitamin D from breast milk

The British Food composition tables (Holland *et al.*, 1991) use the value 0.4 µg/L vitamin D in human breast milk. The same value is used in the Norwegian food composition tables. However, the literature reports a quite large range of concentrations, varying from 0.1 to 1.2 µg/L. A variety of compounds with vitamin D activity (metabolites) are present in human milk, but 25(OH)D accounts for the majority of the antirachitic activity (Reeve *et al.*, 1982; Weisman *et al.*, 1982; Ala-Houhala *et al.*, 1988a; Hillman, 1990). Human milk even from a vitamin D-sufficient mother provides a marginal amount of total vitamin D activity. The 25(OH)D level was higher in hind- than in foremilk (Ala-Houhala *et al.*, 1988a). Vitamin D activity in human milk of unsupplemented mothers was lower in the winter than in the summer. The influence of supplementation with 25 µg ergocalciferol or cholecalciferol or 50 µg cholecalciferol on vitamin D activity in human milk in summer and winter was investigated by Ala-Houhala and co-workers (1988a). They found that supplementation with 50 µg of vitamin D could increase vitamin D activity of milk in the winter to that of unsupplemented mothers in the summer, but the responses varied widely among individuals. Markestad (1983) found a strong correlation between infant and maternal plasma 25(OH)D concentrations both at birth and after 6 weeks in unsupplemented infants born in the winter in the northern areas. The 25(OH)D concentrations in the infants were considerably reduced and reached levels associated with rickets during this period. It appears that sun exposure of the infant is a very important determinant for vitamin D status. Although a study in Caucasians from central USA showed that bone mineralisation was normal in unsupplemented and exclusively breast-fed infants up to 16 weeks (Roberts *et al.*, 1981), most studies agree that fully breast-fed infants have a reduced vitamin D status after 6 weeks of age if no supplemental D is given. The general recommendation therefore is that infants should be supplemented with vitamin D.

2.1.3 Vitamin D intake from food

Only a few foods contain vitamin D, i.e. vitamin D_3, naturally in quantities that have an impact on the dietary intake: fish liver, fish liver oils, fatty fish and egg yolks. Thus, some countries practice fortification of certain foods with vitamin D, most often milk, margarine and/or butter. The mean intakes in different studies vary with age group, food and supplementation habits and gender. Recent publications from various parts of Europe all show that a substantial part of the population including pre-school children has a vitamin D intake below the recommended dietary intakes (Davies *et al.*, 1999; de Jong *et al.*, 1999; Koenig and Elmadfa, 2000; Lehtonen-Veromaa *et al.*, 1999; Ortega *et al.*, 1995; van der Wielen *et al.*, 1995). The low intake is confirmed by results from the SENECA study, an investigation of the diet and health of 824 elderly people from 19 towns in 11 countries (Greece, Portugal, Italy, Spain, France, Switzerland, Hungary, Belgium, Netherlands, Denmark and Norway). Thirty-six per cent of the men and 47% of the women had 25(OH)D concentrations below 30 nmol (van der Wielen *et al.*, 1995). Surprisingly, lowest mean 25(OD)D concentrations were found in southern European countries; more than 80% of Italian and Greek women had values below 30 nmol compared with 18% in Norway. One factor associated with better vitamin D status was increased fish consumption, but the main reasons for the relatively good vitamin D status in the Scandinavian countries are probably fortification of food and a higher percentage of people taking vitamin D supplements. Cod liver oil was taken regularly by 35% of all men and 34% of all women in Norway in 1997, and the percentage was higher among the elderly (Norkost, 1997). A much lower prevalence of vitamin D deficiency was found in the French general adult population; of 1191 adults 11% was below 30 nmol 25(OH)D in serum (Chapuy *et al.*, 1997; Guinot *et al.*, 2000). They found a correlation to latitude and skin exposure, as 24% of those with low exposure was deficient.

The estimated mean dietary vitamin D intakes in several European countries are given in Table 1.

Table 1. The daily intakes of vitamin D (μg/day)

Country	Type of survey	n	Method	Supplements*	Mean	97.5%
Austria[a]	Individual	2488	24h recall	Not defined	4.0	22.2
Germany[b]	Individual (M)	854	7-day dietary	-	4.0	16.8
	Individual (F)	1134	record	-	3.1	11.9
UK[c]	Individual (M)	1087		-	3.4 (2.9)	9.9
	Individual (F)	1110	7-day weighed	-	2.5 (2.2)	6.9
	Individual (M)	1087	inventory	+	3.8 (3.0)	12.7
	Individual (F)	1110		+	3.1 (2.3)	12.6
Italy[d]	Household	2734	7-day record	+	3.0	8.4
Netherlands[e]	Household	5958	2-day record	-	3.7	8.9
Norway[f]	Individual (M)	1298	Semiquantitative	-	5.8	13.0
	Individual (M)	-	FFQ last year,	+	11.2	37.6
	Individual (F)	1374	180 food items	-	4.0	10.3
	Individual (F)	-		+	10.3	33.3
Ireland[g]	Individual (M)	662	7-day estimated	+	3.7	13.5
	Individual (F)	717	food record	+	3.7	14.9

* + data included supplements; - data excluded supplements.

[a] Elmadfa *et al.* (1998). [b] Heseker *et al.* (1994) - values are the median.

[c] Gregory *et al.* (1990) - values are the mean with the median in parentheses. [d] Turrini (1996).

[e] Hulshof and Kruizinga (1999). [f] Norkost (1997). [g] IUNA (2001).

2.2 Metabolism of vitamin D

2.2.1 Vitamin D activation

Both forms of vitamin D (vitamin D_3, cholecalciferol, and vitamin D_2, ergocalciferol) are inactive. Major metabolic steps involved in the metabolism of vitamin D_2, mono and dihydroxylated forms, are similar to those of vitamin D_3. Vitamin D without a subscript represents either D_2 or D_3 or both and requires two obligate hydroxylations to form the active hormone, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). The first step of activation takes place by hydroxylation at position C-25, mainly in the liver. The role of other tissues is uncertain. The product, 25-hydroxyvitamin D (25(OH)D), is transported to the kidneys, where 1α-hydroxylation takes place. The resulting product, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) , is the active metabolite. $1,25(OH)_2D$ is transported bound to vitamin D-binding protein (DBP). DBP is synthesised in the liver and circulates in plasma at concentrations 20 times higher than the total amount of vitamin D metabolites. The role of the large molar excess of DBP is uncertain. Free $1,25(OH)_2D$ is in equilibrium with the bound form. It is only free $1,25(OH)_2D$, i.e. 0.5% of the total amount of plasma $1,25(OH)_2D$, which is hormonally active. The binding to DBP increases the half-life of $1,25(OH)_2D$ and makes the hormone available to the cells (Brown *et al.*, 1999). The concentration of DBP is increased during pregnancy and by oestrogen treatment. It also increases in infants after birth.

The 25-hydroxylation of vitamin D is poorly regulated, i.e. the capacity of the 25-hydroxylase in the liver is high. The levels of 25(OH)D increase in proportion to vitamin D intake, and for this reason, plasma 25(OH)D levels are commonly used as indicator of vitamin D status. The half-life of 25(OH)D in circulation is approximately 1-2 months (Vieth, 1999). Steady state in plasma 25(OH)D concentration would, according to the half-life, not be reached before 4 months after a change in the intake. With concentration-dependent kinetics this could, however, vary. The proportion of 25(OH)D to vitamin D intake cannot be determined before steady state is reached.

The serum level of 25(OH)D usually reflects both $25(OH)D_3$ and $25(OH)D_2$. The ratio of these two hydroxylated derivatives depends on the relative amounts of vitamins D_2 and D_3 present in the diet and endogenously synthesised vitamin D_3 (Holick *et al.*, 1998).

In contrast, the production of $1,25(OH)_2D$ is tightly regulated, both by feedback of the $1,25(OH)_2D$, through calcium and phosphate levels in the blood and with the help of parathyroid hormone (PTH). This is illustrated by experiments showing that when large doses of vitamin D are given to animals, the serum concentrations of 25(OH)D will increase proportionally, while the concentration of $1,25(OH)_2D$ remains normal. Both the suppression of the kidney 1α-hydroxylase activity and induction of the 24-hydroxylase activity are VDR-mediated. Experiments with rats have shown that tissue specific down-regulation of renal VDR by calcium restriction blocks $1,25(OH)_2D_3$-dependent suppression of renal 1α-hydroxylase or stimulation of renal 24-hydroxylase (Brown *et al.*, 1999; Beckman and DeLuca, 2002).

2.2.2 Catabolism of vitamin D

The major catabolic enzyme is the 24-hydroxylase, a mitochondrial enzyme, and both 25(OH)D and $1,25(OH)_2D$ are inactivated via this pathway. Further oxidation to the ketone, oxidation at C-23(S) and C-26, and subsequent oxidative cleavage of the side chain are

associated with progressive loss of biological activity. Also additional pathways have been described (Brown *et al.*, 1999).

In contrast to the limited distribution of the vitamin D-activating enzymes, 24-hydroxylase is ubiquitously present in vitamin D target tissues. This enzyme is highly inducible by $1,25(OH)_2D$ providing a regulatory mechanism at the cellular level for attenuating the response of the active compound when abnormally high.

2.3 Functions of vitamin D

The principal function of vitamin D ($1,25(OH)_2D$) in the body is to maintain intracellular and extracellular calcium concentrations within a physiologically acceptable range. The vitamin accomplishes this goal through the action of $1,25(OH)_2D$ on regulating calcium and phosphorus metabolism in the intestine and bone.

2.3.1 Vitamin D receptor (VDR)

The main mechanism of action of vitamin D is the interaction of $1,25(OH)_2D$ with the nuclear vitamin D receptor (Brown *et al.*, 1999). VDR belongs to the super family of steroid nuclear receptors. Following ligand binding, VDR heterodimerises with retinoid X receptor (RXR) and acts as a ligand-activated transcription factor by binding to genomic vitamin D responsive elements (VDRE) in vitamin D-regulated genes. These include more than 50 other genes important for mineral homeostasis, vitamin D metabolism, energy metabolism, cell differentiation and proliferation, extracellular matrix proteins, oncogenes, growth factors, signal transduction proteins and peptide hormones. Genes can be both up-regulated or down-regulated, but the exact mechanism is unclear. Among genes down-regulated are PTH, osteocalcin, protein-kinase A inhibitors and interleukin-2 genes.

Several genetic polymorphisms of VDR have been identified, the exact role of these has not been clarified, but most variants do not affect the protein structure (Brown *et al.*, 1999). In a study on the efficacy of vitamin D supplementation on bone mineral density of the femoral neck in elderly women it was found that those having one or two *VDR* alleles without the BsmI restriction site responded better than those with a genotype in which this restriction site was absent (Graafmans *et al.*, 1997).

The cellular response to 1,25(OH)D is mainly regulated by changing the cellular amount of VDR. Treatment with 1,25(OH)D increases the receptor level presumably due to stabilisation of the receptor. Some growth factors increase, as IGF-I, while others, such as fibroblast growth factor and mitogens, decrease VDR expression. Activation of protein-kinase C and prednisone treatment inhibit VDR expression whereas oestrogen, retinoic acid and PTH increase VDR expression. VDR expression is also dependent on cell type, and its condition, proliferating or differentiating (Kveiborg *et al.*, 1999). VDR can also be regulated at the stage of degradation. VDR interacts directly with SUG1, a component of the proteasome complex important for proteolysis. VDR activity might also be modulated by phosphorylation of serine at different positions (Brown *et al.*, 1999). VDR knockout mice have been produced. The homozygous mouse ($VDR^{-/-}$) shows no sign of defect until end of weaning. Then they fail to thrive and die within 15 weeks from birth. They suffer from hypokalaemia, defective fur and females have defects in reproductive organs. Furthermore, bone formation and growth are inhibited and the level of 1,25(OH)D is increased indicating a role of VDR in regulation of vitamin D hydroxylation. In some respects the VDR knockout mice show some phenotypic

similarities with the disease vitamin D resistant-rachitis type 2, which is seen in children with inherited mutations in VDR (Yoshizawa *et al.*, 1997; Kveiborg *et al.*, 1999).

2.3.2 Vitamin D and calcium homeostasis

The most critical role of $1,25(OH)_2D$ in mineral homeostasis is to enhance the efficiency of the small intestine to absorb dietary calcium. This was clearly demonstrated in the VDR null mouse (Yoshizawa *et al.*, 1997). Calcium absorption from the intestine is dependent on the amount of calcium in the diet and on physiological requirements, and is adaptable. When dietary calcium concentrations are low, almost all calcium is absorbed. The same happens in pregnancy and during lactation. $1,25(OH)_2D$ also promotes the intestinal absorption of phosphate. However a significant phosphate absorption also occurs in $1,25(OH)_2D$-deficient states (Brown *et al.*, 1999).

$1,25(OH)_2D$ is essential for development and maintenance of a mineralised skeleton. Deficiency results in rickets during growth and osteomalacia in adults. $1,25(OH)_2D$ induces bone formation by regulation of matrix proteins important for bone formation, such as osteocalcin, osteopontine, alkaline phosphatase, matrix-gla- protein and collagen, as well as mineral apposition. The bone forming osteoblasts express VDR and it appears that $1,25(OH)_2D$ inhibits osteoblast proliferation through VDR-dependent signal pathway, and promotes their differentiation (Kveiborg *et al.*, 1999). Vitamin D does not appear to be absolutely essential for the ossification process, but enhances this through increasing serum levels of calcium and phosphate. It has been suggested that not only $1,25(OH)_2D$ is involved in bone mineralisation, but also $24,25(OH)_2D$ may be required (Brown *et al.*, 1999).

$1,25(OH)_2D$ enhances the mobilisation of calcium and phosphorus stores from bone at times of calcium deprivation. $1,25(OH)_2D$ induces stem cell monocytes to become mature osteoclasts. It appears though that this effect is not direct, but is mediated via osteoblasts that secrete a factor promoting osteoclast differentiation (Kveiborg *et al.*, 1999). $1,25(OH)_2D$ regulate calcium homeostasis in close co-operation with PTH, which is the principal hormone regulating extracellular ionised calcium from minute to minute. PTH stimulates $1,25(OH)_2D$ synthesis and $1,25(OH)_2D$ suppresses the synthesis and secretion of PTH and controls parathyroid growth through negative gene regulation. Studies in the VDR null mouse suggest that VDR is not essential, but works in co-operation with calcium and phosphate (Brown *et al.*, 1999).

The most important effects of $1,25(OH)_2D$ in the kidney is suppression of 1α-hydroxylase activity and induction of 24-hydroxylase activity. $1,25(OH)_2D$ increases renal calcium reabsorption and calbinding expression, and it accelerates PTH dependent calcium transport in the distal tubule, which has the highest level of VDR. The enhancing effect of $1,25(OH)_2D$ on renal phosphate absorption might be an indirect action via PTH suppression (Brown *et al.*, 1999).

2.3.3 Other effects of vitamin D

Synthesis and cellular receptors for $1,25(OH)_2D$ have been found not only in the intestine, kidney and bone but also in many other tissues, suggesting that $1,25(OH)_2D$ is fundamental to the regulation of gene expression in many cell types in addition to its probable role in intracellular calcium regulation (Brown *et al.*, 1999; Zehnder *et al.*, 2002a and b). Further local production and action of $1,25(OH)_2D$, particularly after inflammatory activation of 1α-hydroxylase activity by, for example, cytokines in endothelial cells, could indicate an

important autocrine/paracrine mechanism in peripheral tissues (Zehnder *et al.*, 2002b). Addition of $1,25(OH)_2D_3$ or $25(OH)D_3$ decreased proliferation of human endothelial cells and the adhesion of monocytic cells to these cells (Zehnder *et al.*, 2002b). In the skin, $1,25(OH)_2D$ plays an important role by inhibiting proliferation and stimulating differentiation of keratinocytes and vitamin D analogues are used in the treatment of psoriasis. In the immune system, $1,25(OH)_2D$ modulates synthesis of interleukins and cytokines. Besides stimulating monocytes and macrophages, $1,25(OH)_2D$ functions as an immunosuppressive agent by decreasing the rate of proliferation and the activity of both T- and B cells and inducing suppressor T cells (Brown *et al.*, 1999). In haematopoietic tissue, vitamin D deficiency causes anaemia and decreased cellularity of bone marrow. $1,25(OH)_2D$ also inhibits proliferation and promotes differentiation of a number of leukaemia cell lines. Also normal myeloid precursor cells mature in the presence of $1,25(OH)_2D$. In addition, VDR is expressed in many other tissues, such as muscle and nervous tissue, liver, intestine, reproductive organs, pancreas, pituitary, thyroid gland and lung, where $1,25(OH)_2D$ apparently has important functions in regulation of cell proliferation and differentiation (Brown *et al.*, 1999; Holick, 1999). In animal experiments and also in epidemiological studies, vitamin D appears to be a protective factor in colon carcinogenesis.

2.3.4 25(OH)D and the vitamin D receptor

At very high levels it appears that 25(OH)D also has a direct effect on the vitamin D receptor. However, another possible action of 25(OH)D might also operate (Vieth, 1990). When excess vitamin D is consumed there is increased and uncontrolled formation of 25(OH)D, which is secreted from the liver into blood. The specific vitamin D-binding sites on the circulating vitamin D-binding protein, which normally is less than 5% saturated, become mainly occupied with 25(OH)D. Because $1,25(OH)_2D$ has a lower affinity for this protein than 25(OH)D, the functional hormone is displaced and circulates either in the unbound form or in loose association with plasma albumin. Hence the availability of $1,25(OH)_2D$ to its intracellular receptors is greatly increased by the swamping of vitamin D-binding protein with 25(OH)D. Furthermore, because the intracellular receptors have a much higher affinity for $1,25(OH)_2D$ than does vitamin D-binding protein, they will readily take up this displaced $1,25(OH)_2D$ from extracellular fluid. Although the production of $1,25(OH)_2D$ may not be increased in hypervitaminosis D, its supply to sites of action will, in this way, be greatly raised.

2.3.5 Non-vitamin D receptor-mediated effects

In addition to VDR-mediated effects, $1,25(OH)_2D$ apparently also elicits rapid cellular responses by interacting with specific cell surface receptors giving rise to rapid changes in phosphoinositide metabolism and increases in intracellular calcium levels, stimulating intestinal calcium and phosphate fluxes. The receptor has only partially been characterised and the role of non-genomic actions of $1,25(OH)_2D$ in most cells remains unclear.

2.4 Biomarkers

2.4.1 Biomarkers of vitamin D intake

Plasma derivatives of vitamin D_2 ($25(OH)D_2$) are of exogenous origin only, while derivatives of vitamin D_3 ($25(OH)D_3$) may arise from either diet or skin. Different studies get very different correlations between vitamin D dietary intake and serum levels of 25(OH)D, and the reason is obvious: the amount of 25(OH)D originating from sun exposure will confound all

attempts to use 25(OH)D as a biomarker of dietary intake alone. Thus 25(OH)D can only be used as a biomarker of vitamin D intake in people whose sunshine exposure has been low. Furthermore not all studies on vitamin D supplementation have determined 25(OH)D when in a steady state condition.

It should be noted that 25(OH)D denotes both the D_2 and D_3 metabolites and it is not always reported in publications if total 25(OH)D has been measured or either one. Furthermore there may be a variation between laboratories of more that 30%, making comparisons of exact values from different studies, particularly old ones, difficult (Lips *et al.*, 1999).

2.4.2 Biomarkers of vitamin D status and activity

There is now consensus that serum 25(OH)D concentration is a good marker of internal vitamin D status. In patients with hypervitaminosis D, serum 25(OH)D levels are 2-15 times higher than those of normal controls (Hughes *et al.*, 1976; Vieth, 1999). Hypercalcaemia frequently coexists with high levels of 25(OH)D and is a useful marker of hypervitaminosis D. In addition PTH will be suppressed.

Plasma levels of $1,25(OH)_2D$, and particularly free $1,25(OH)_2D$, is a measure of vitamin D hormone activity, but because of its tight regulation it does not reflect very well vitamin D nutritional status.

2.4.3 Reference serum levels of metabolites

Most diagnostic laboratories consider the upper reference level of 25(OH)D in serum to be about 150 nmol/L (Holick, 1999), i.e. 130-150 nmol/L. The reference levels for $1,25\ (OH)_2D$ is 50-145 pmol/L and for $24,25(OH)_2D$ is 2-10 nmol/L. The reference values of 25(OH)D in infants, 130-150 nmol/L, are similar or close to those of adults (Markestad, 1984). PTH is raised in vitamin D deficiency and should also be determined to establish this diagnosis.

2.5 Endogenous synthesis in the skin of vitamin D and nutritional requirement

Exposure of the skin to solar ultraviolet B with energies between 290 and 315 nm catalyses the conversion of 7-dehydrocholesterol to previtamin D_3 (precholecalciferol), which spontaneously isomerises to cholecalciferol (Holick, 1995). Upon prolonged UV exposure a regulation mechanism is operating in that both precholecalciferol and cholecalciferol can be photolysed to inert compounds. Hence, sunlight alone apparently cannot cause overt toxicity due to overproduction of vitamin D. Even though the skin phototype in a study from France (Guinot *et al.*, 2000) did not influence vitamin D status, other studies indicate that the degree of pigmentation of the skin also has an impact on the amount of vitamin D synthesised as melanin absorbs UV B photons: the darker the skin, the less is produced. Skin thickness decreases linearly with age from the age of 20 years and there is a marked decrease in the precursor 7-dehydrocholesterol in the skin and less vitamin D production. When healthy young and old men were compared after exposure to UV light, young men had nearly 4 times more circulating 25(OH)D in serum than the old individuals (Need *et al.*, 1993; Holick, 1995). The concentrations of 25(OH)D in serum tend to decrease with age.

The vitamin D requirement for healthy adults has never been defined precisely. Because vitamin D is produced in the skin upon exposure to sunlight, humans, with the possible exception for elderly, do not have any requirement for vitamin D when sufficient sunlight is available. However, vitamin D becomes an important nutritional factor in the absence of

sunlight and in the elderly. It is well known that a substantial part of the European population is exposed to sub-optimal levels of sunlight, especially during the winter months. In addition to geographical and seasonal factors, exposure to sunlight is dependent on modern life style such as clothing and indoor life (McKenna, 1992).

There is now a consensus that serum 25(OH)D concentration is the correct functional indicator of vitamin D status, which is also used as a basis for the nutritional recommendations (FNB, 1999; Vieth *et al.*, 2001). A level of 25(OH)D below 27.5 nmol/L is considered to be consistent with vitamin D deficiency in infants, neonates and young children (Specker *et al.*, 1992). This value should be met to prevent rickets and severe osteomalacia in these groups (FNB, 1999). Little information is available about the level of 25(OH)D needed to maintain normal calcium metabolism and peak bone mass in adolescents and middle aged adults. For elderly there is increasing evidence of a greater requirement of vitamin D to maximise bone mineralisation. Less certain and more controversial is the optimal serum concentration of 25(OH)D. Moderate vitamin D malnutrition is based on the now well documented inverse relationship between serum concentrations of 25(OH)D and PTH (Vieth *et al.*, 2001). A serum concentration of 25(OH)D <40-50 nmol/L is considered by several authors to be insufficient, particularly in the elderly with bone loss, and many regard serum 25(OH)D concentrations above 75-100 nmol/L to be desirable, concentrations at which PTH is suppressed to a minimum in its relation to 25(OH)D (Chapuy *et al.*, 1997; Chel *et al.*, 1998; Dawson-Hughes *et al.*, 1997; Gallagher *et al.*, 1998; Kinyama *et al.*, 1998; Thomas *et al.*, 1998; Vieth, 1999; Vieth *et al.*, 2001).

2.5.1 Existing recommendations on vitamin D intake

The necessary intake of vitamin D will depend on the shortfall of exposure to effective UV radiation. Most countries have their own recommendations for vitamin D intake, recognising that there may be insufficient sun exposure in larger or smaller groups of the population. Term infants are born with a store of vitamin D reflecting the mother's vitamin D status. These stores provide the infant with sufficient vitamin D for 4-6 weeks. The vitamin D content of mothers' milk from women living in industrialised societies is not considered sufficient to maintain adequate vitamin D status in the child. Thus, many countries recommend 10 µg vitamin D/day to infants from 4 weeks onwards. The same amount is recommended for pregnant and lactating women. The current allowance of vitamin D recommended by most European countries is 5 µg/day (200 IU) for adults and 10 µg vitamin D per day for everyone older than 60-65 years. The separate European countries often have more detailed recommendations than the general ones mentioned here, and the recommended values vary somewhat (Trichopoulou and Vassilakou, 1990). The Population Reference Intake (PRI) recommended by the Committee (SCF, 1993) are as follows: 6-11 months 10-25 µg; 1-3 years 10 µg; 4-10 years 0-10 µg; 11-17 years 0-15 µg; 18-64 years 0-10 µg; ≥65 years 10 µg; pregnancy 10 µg; lactation 10 µg.

2.5.2 Differences in metabolism and bioefficiency of different forms of vitamin D and effect of vehicle

Based on studies in the 1930s showing no conclusive difference between vitamin D_3 (from cod liver oil) and D_2 in their preventing effect against infantile rickets, vitamin D_2, for practical purposes, has been regarded as equal to vitamin D_3 from cod liver oil (Trang *et al.*, 1998). However, in several non-human species vitamin D_3 and D_2 show differences in their ability to increase 25(OH)D (Marx *et al.*, 1989). Also in the pig and birds vitamin D_3 is far more effective than D_2, whereas the opposite is the case in rats (Horst *et al.*, 1982). Although

very early studies did not show differences in antirachitic activity between vitamin D_2 and D_3, more recent studies (Tjellesen *et al.*, 1985; Hartwell *et al.*, 1987 and 1989), including a study by Tjellesen *et al.* (1986) in premenopausal women, did show a greater efficacy with vitamin D_3 in humans. This issue was recently addressed in a larger study by Trang *et al.* (1998). They were able to show that vitamin D_3 was 1.7 times as efficient as D_2, when given in equimolar amounts during 14 days to healthy volunteers, to raise the serum level of total 25(OH)D. Particularly, higher basal level of 25(OH)D supplementation with high levels of vitamin D_2 was inefficient. Studies have also shown that vitamin D_2 supplementation can suppress endogenously formed $25(OH)D_3$ and also $1,25(OH)_2D_3$ (Tjellesen *et al.*, 1986; Hartwell *et al.*, 1989; Harris *et al.*, 1999).

Studies during the last decade have revealed that the differences in the side chain between vitamin D_3 and D_2 result in differences in hydroxylated products particularly when large doses are administered (Mawer *et al.*, 1998). Direct 24-hydroxylation of vitamin D_2 in the liver is of particular relevance since routing of vitamin D_2 to $24(OH)D_2$ would lead to further inactivation or via the kidney to the biologically active $1\alpha,24(OH)_2D_2$. Interestingly however, $1\alpha,24(OH)_2D_2$, which is a significant metabolite at high doses of vitamin D_2, binds strongly to VDR and possesses potent antiproliferative activity in combination with low calcaemic activity (Jones *et al.*, 1996; Knutson *et al.*, 1997; Mawer *et al.*, 1998). $1\alpha,24(OH)_2D_2$ can also form from $1\alpha(OH)D_2$, which is less toxic than $1\alpha(OH)D_3$ (Knutson *et al.*, 1997; Sjoden *et al.*, 1985).

The observed reduced ability of vitamin D_2 to raise plasma 25(OH)D and the low calcaemic effect of $1\alpha,24(OH)D_2$ formed at high doses of vitamin D_2 including suppressing effect on $25(OH)D_3$ and $1\alpha,25(OH)_2D_3$ synthesis are most probably the reasons for a lower toxicity of vitamin D_2 than of vitamin D_3 as the toxic effects are mainly related to the distorted calcium metabolism (see below).

The vehicle used (fat or emulsion) in which vitamin D is administered could influence bioavailability. This was shown as early as in 1935 by Stearn and Jeans (cited in Seelig, 1969). Vitamin D from cod liver oil emulsified in milk is about three times as bioavailable as judged by potency as vitamin D given in cod liver oil or propylene glycol.

3. HAZARD IDENTIFICATION AND CHARACTERISATION

3.1 Mechanisms of toxicity

The toxic effects of vitamin D excess are primarily related to the role of free $1,25(OH)_2D$ in the regulation of plasma calcium (Davies and Adams, 1978; Reichel *et al.*, 1989). Excessive production of $1,25(OH)_2D$ or greatly increased plasma 25(OH)D (which may displace $1,25(OH)_2$ D from DBP) may lead to elevated level of plasma calcium due partly to over-stimulated intestinal absorption and partly to excessive calcium mobilisation from bone (Norman, 1996; Pettifor *et al.*, 1995; Vieth, 1990). Hypercalcaemia could also lead to an increased calcium excretion into urine, hypercalciuria. There is also limited evidence that high concentrations of vitamin D directly affect various organ systems such as kidney, bone, the central nervous system and the cardiovascular system (Holmes and Kummerow, 1983).

Hypercalcaemia is defined as a serum calcium above 2.75 mmol/L or ionised calcium above 1.35 mmol/L. Hypercalcaemia associated with hypervitaminosis D gives rise to numerous debilitating effects (Chesney, 1990; Holmes and Kummerow, 1983; Parfitt *et al.*, 1982).

Specifically this would include loss of tubular concentration function of the kidney with polyuria and hypercalciuria, which would predispose to nephrolithiasis and reduced glomerular filtration rate. Prolonged hypercalcaemia can cause calcification of soft tissues, including kidney, blood vessels, heart and lungs (Allen and Shah, 1992; Moncrief and Chance, 1969; Taylor *et al.*, 1972). A 24-hour urinary calcium excretion >10 mmol is considered to indicate hypercalciuria. The mean molar calcium/creatinine ratio in randomly collected urine from non-fasting healthy subjects is approximately 0.40. The relation between this ratio in urine and the 24-hour calcium excretion indicate that 10 mmol Ca/24 hours would correspond to a ratio in urine of about 1.0 in molar calcium/creatinine. Whether a high calcium excretion in a human with serum calcium within reference limits should be regarded as an adverse effect is not clear. In the absence of hypercalcaemia and low urine volume urinary calcium *per se* is a minor contributor to renal stone disease (Vieth *et al.*, 2001).

3.2 Genotoxicity

Vitamin D_3 was tested in the *Salmonella typhimurium* assay at doses 0.033 to 10 mg/plate in *Salmonella typhimurium* (strains TA1535, TA1537, TA97, TA98 and TA100) in the absence and presence of rat or hamster liver S9. Vitamin D_3 was negative in these tests. Doses above 1 mg/plate exhibited slight toxicity (Mortelmans *et al.*, 1986).

No studies using other test systems for genotoxicity either *in vitro* or *in vivo* have been identified.

3.3 Acute toxicity

3.3.1 Animal data

The lethal dose in dog is said to be 13 mg/kg body weight. Immediate effects are bloody diarrhoea, anorexia, thirst, polyuria and prostration. In surviving animals calcium is deposited as in chronic hypervitaminosis D (Clare and Clark, 1975).

3.3.2 Human data

3.3.2.1 Effects of single doses

Elderly subjects with a serum calcium <2.75 mmol/L tolerated well a single intramuscular dose of 7,500 µg of vitamin D_2 when given once a year for 4 years (Heikinheimo *et al.*, 1992 and 1991). Measurements of serum calcium just after the injection were not reported, excluding detection of possible transient hypercalcaemia. Serum calcium was marginally elevated 2-3 months after the injection. Coles *et al.* (1985, cited in Heikinheimo *et al.*, 1992) used 10,000 µg vitamin D intramuscularly with no apparent toxic effects.

The safety of vitamin D prophylaxis as “stosstherapie” in infants 1-2 years was investigated by Markestad *et al.* (1987). An oral dose of 15,000 µg ergocalciferol (vitamin D_2) was given every 3-5 months. Calcium, phosphorous and vitamin D metabolites were measured before and 2 weeks after each dose. 25(OH)D increased to median concentrations between 240 to 430 nmol/L (ranges: 130-930 nmol/L) (data extracted from figure) and returned to levels below 130 nmol/L before the next dose. All infants had normal serum calcium levels before the first dose, but 14 infants (34%) had calcium levels above 2.80 mmol/L (2.81-3.32 mmol/L), indicating that the vitamin D doses were excessive despite the lack of accumulative increases in 25(OH)D concentrations. In a later study (Misselwitz *et al.*, 1990) ten children in

the age range 1½ to 14 years, who had received such treatment, were diagnosed to have nephrocalcinosis. At the time of investigation, however, their serum vitamin D status was normal. This would indicate that even recurrent transient episodes of vitamin D excess and hypercalcaemia could lead to irreversible toxic effects as, for example, nephrocalcinosis.

In a study using vitamin D_3 (cholecalciferol) in oral doses of 15, 5 or 2.5 mg every 3 months, Zeghoud *et al.* (1994) showed that these doses gave 25(OH)D concentrations of 307±160, 150±55, and 92±42 nmol/L, respectively, two weeks after the first dose. Serum calcium transiently increased 2 weeks after 15 mg, but not after the lower doses. Prolonged vitamin D overload, up to 6 months was seen in 50% of the children given the highest dose.

A single episode of moderately severe hypercalcaemia in infants may arrest growth for several months (Haynes, 1990).

3.4 Reproduction

3.4.1 Animal data

Vitamin D has been found to be teratogenic in animals at 4-15 times the recommended human dose. Offspring from pregnant rabbits treated with such high doses of vitamin D had lesions anatomically similar to those of supravalvular aortic stenosis and offspring not showing such changes show vasculotoxicity similar to that of adults following acute vitamin D toxicity (Stockton and Paller, 1990). The symptoms are most likely due to hypercalcaemia.

Sows received diets containing either 55 or 8.15 µg vitamin D_3 per kg basal ration (equivalent to 3.4 or 0.5 µg/kg body weight) and 6 week-old piglets were examined for coronary arterial lesions. Piglets from sows fed the high vitamin D_3 diet had more degenerated smooth muscle cells than those fed the low dose (Toda *et al.*, 1985b).

3.4.2 Humans

During pregnancy 25(OH)D in maternal serum correlates with vitamin D intake, whereas the circulating active metabolite $1,25(OH)_2D$ is elevated mainly due to synthesis in the decidual cells of the placenta. Also the binding protein (DBP) increases. The foetus is entirely dependent upon maternal supply of 25(OH)D, which together with $24,25(OH)_2D$ appears to diffuse easily across the placenta. The relationship between $1,25(OH)_2D$ concentrations in maternal and foetal circulation is more complex as some studies show a good correlation whereas others do not (Salle *et al.*, 2000).

There are also reports on $1,25(OH)_2D$ treatment during pregnancy of women suffering from hypoparathryoidism (Salle *et al.*, 1981) (dose 0.5-2 µg/day) or insensitivity to $1,25(OH)_2D$ (dose 17-36 µg/day) (Marx *et al.*, 1980). In the latter case the mother had extremely high plasma $1,25(OH)_2D$ and normocalcaemia. At parturition the cord serum concentration of $1,25(OH)_2D$ was strongly elevated, 940 pmol/L (normal mean: 47.5 pmol/L) and the child had mild hypercalcaemia the first two days of life. None of the children had other signs of toxicity. This indicates a minor impact of circulating $1,25(OH)_2D$ on calcium levels *in utero*. This is further supported by the fact that supplementary vitamin D (25 µg/day) during the last trimester reduced the fraction of infants displaying growth retardation (Salle *et al.*, 2000).

However, maternal hypercalcaemia during pregnancy may increase foetal sensitivity to effects of vitamin D, suppression of parathyroid function or a syndrome of elfin faces, mental

retardation, and congenital supravalvular aortic stenosis. There are, however, no controlled studies in pregnant women indicating at which doses this may occur (Haynes, 1990).

Maternal supplementation of lactating women with 25 and 50 µg vitamin D_2/day during winter time showed that only children of women supplemented with the highest dose normalised the concentration of circulating vitamin D metabolites. Infants who got 10 µg vitamin D/day supplement and were breast-fed by non-supplemented mothers had similar vitamin D status to those of mothers supplemented with the highest dose (Ala-Houhala *et al.*, 1986).

3.5 Chronic toxicity

3.5.1 Animal data

Hypervitaminosis D in animals as in humans is associated with hypercalcaemia and adverse effects largely mediated by this condition. The severity of the symptoms and organ manifestations depend on the severity and length of the hypercalcaemia. Soft tissue calcifications are common effects.

Charles River Crl:CD BR rats were given daily doses of 0, 12.5, 25 and 50 µg vitamin D_3/kg body weight from 10 weeks of age (Tischler *et al.*, 1999). All doses of vitamin D_3 markedly increased serum calcium and phosphorus levels and calcium excretion into urine. At 4 weeks the rats receiving 12.5 and 25 µg vitamin D_3/kg body weight/day showed occasional foci of kidney tubular calcification while this was more prevalent at the highest dose of 50 µg vitamin D_3/kg body weight. At 26 weeks all kidneys from the highest dose showed mild to moderate nephrocalcinosis, the rats receiving 25 and 12.5 µg vitamin D_3/kg body weight/day showed mild and nearly no calcinosis, respectively.

Groups of two month-old swine were fed dietary vitamin D_3 at doses of 2.5, 7.5, 50, and 100 µg/kg feed (equivalent to 0.15, 0.45, 3 and 6 µg vitamin D/kg body weight, respectively) for four months. Particularly the highest dose group had thickening of the intima of the coronary vessels. Increased levels of lipid containing- and degenerative cells were also seen (Toda *et al.*, 1985a)

3.5.2 Symptoms of vitamin D intoxication in humans

The symptoms of hypervitaminosis D are connected with the physiological consequences of hypercalcaemia, which occur once the calcium eliminating capacity of the kidneys is exceeded. The most frequently noted clinical manifestations of hypervitaminosis D are anorexia, weight loss, weakness, fatigue, disorientation, vomiting and constipation (Blank *et al.*, 1995). Hypercalcaemia may also lead to growth retardation in children, irritability, asthenia, persisting fever, polyuria and polydipsia, dehydration, hypertension and functional renal insufficiency. Long-term toxicity with persistent hypercalcaemia may cause excess calcium precipitates as extra-skeletal calcium in soft tissues, particularly in the renal parenchyma, urinary tracts, vascular walls, muscles and tendons.

Linden (1974) observed that myocardial infarct patients in Tromsø, Norway, were more likely to consume vitamin D in excess of 30 µg/day than were matched controls, but two subsequent studies (Schmidt-Gayk *et al.*, 1977; Vik *et al.*, 1979) failed to confirm this.

Further studies are needed to clarify progressive health effects of regular and moderately high amounts of vitamin D over several decades.

3.5.3 Serum 25(OH)D and vitamin D toxicity

Vieth (1999) summarised the dose-response for mean vitamin D intake *versus* final serum 25(OH)D concentration of supplemented groups from 35 reports. Many of the studies involved not more than 4 weeks of supplementation, and according to the half-life for 25(OH)D of 1 to 2 months, one would not assume steady state to be achieved in such a short time. Remarkably, serum level of 25(OH)D concentration is maintained within a narrow range, ≈75-220 nmol/L across vitamin D supplies from 20 μg/day up to 250-500 μg/day. Beyond this level of vitamin D intake, which may be the physiologic limit, there is a classical rise in the dose-response curve associated with toxicity. Apparently there are homeostatic control systems to regulate serum 25(OH)D and to buffer against variability in vitamin D supply. Interestingly, this physiological limit of vitamin D intake is comparable with the amount of vitamin D (250-625 μg/day) estimated to be produced by full-body exposure to sunlight (Stamp, 1975; Holick, 1995).

A patient who had received vitamin D as a single monthly dose of 7,500 μg for several months had a serum 25(OH)D level of about 600 nmol 25(OH)D/L and experienced toxicity (Rizzoli *et al.*, 1994). The dose was toxic since the production of 25(OH)D apparently had exceeded the instant capacity of the homeostatic control system. Exposure to a single large dose of vitamin D resulted in a rapid and high peak in serum 25(OH)D concentration, with concentrations falling progressively thereafter (Davie *et al.*, 1982; Weisman *et al.*, 1986).

Barger-Lux *et al.* (1998) administered 25, 250 or 1250 μg cholecalciferol/day to young healthy men with a mean serum 25(OH)D of 67 nmol/L. After 8 weeks serum 25(OH)D increased by 29, 146 (100-225) and 643 (400-1000) nmol/L for the three dosage groups. Body mass index (BMI) and not weight contributed significantly to the variance in 25(OH)D response. A high BMI would predict less change in 25(OH)D upon supplementation. The treatment time in this study could have been too short to achieve steady state level of 25(OH)D.

Himmelstein and coworkers (1990) supplemented elderly with 50 μg cholecalciferol/day in a double blind study for six weeks. At week 7 the serum concentration of 25(OH)D had reached 80.1±6.7 nmol/L. It cannot be excluded that a plateau had not been reached.

Davie and co-workers (1982) gave 10, 25 and 250 μg vitamin D/day for 2.5 months. The two lowest doses reached a plateau of about 55 nmol 25(OH)D whereas the highest dose, 250 μg/day, reached a serum level of about 120-140 nmol/L. However, it is likely that steady state had not been reached in this case, furthermore the form of vitamin D is uncertain.

When Stamp *et al.* (1977) measured 25(OH)D in 128 individuals receiving the same daily amount of vitamin D_2 or D_3 for a period more than 4 months and having achieved steady state, those receiving 45 μg/day were all below 130 nmol 25(OH)D/L, whereas among those receiving 150 μg/day a large fraction had values above 130, but less than 200 nmol/L. The upper 95% confidence limit for the regression line crossed 130 nmol/L at about 60-70 μg/day. (All the data were extracted from the figures).

Vieth and coworkers (2001) supplemented two groups of 33 and 28 healthy volunteers with 25 and 100 μg cholecalciferol daily, respectively, for 1-5 months. At 4 and 5 months the lower dose group had a mean 25(OH)D concentration of about 70 nmol/L (range: 45-120) and in the high dose group the mean was about 100 nmol/L (range: 65-120) (values extracted from figure).

Tjellesen *et al.* (1986) supplemented 19 healthy premenopausal women with either 100 μg ergocalciferol or cholecalciferol/day for eight weeks. They also received 0.5 g calcium per day. At eight weeks the total 25(OH)D concentrations in serum were 35.5 (19.7-48.3) and 45.4 (31.0-55.4) nmol/L in the ergocalciferol and cholecalciferol group, respectively. A suppression of $25(OH)D_3$ in serum was observed in the group treated with ergocalciferol resulting in no change in the total 25(OH)D from the pre-treatment status.

3.5.4 Vitamin D intake and hypercalcaemia

Hypercalcaemia is defined as a serum calcium level above 2.75 mmol/L or ionised calcium above 1.35 mmol/L. Normal calcium levels were seen in persons given 50 μg/day of vitamin D for 6 months (Johnson, 1980) and daily intake for 6 weeks of 250 μg by healthy adults did not significantly raise their serum and urine concentrations of calcium (Berlin *et al.*, 1986). In individuals with intakes from 1250 μg/day or higher the serum calcium level range was from 2.82 to 4.00 mmol/L (Schwartzman and Franck, 1987; Davies and Adams, 1978; Selby *et al.*, 1995; Rizzoli *et al.*, 1994; Pettifor *et al.*, 1995). Schwartzman and Franck (1987) reviewed cases in which vitamin D was used to treat osteoporosis in middle aged and elderly women. These women had health problems in addition to osteoporosis. An intake of vitamin D between 1250 μg/week and 1250 μg/day for 6 weeks to 5 years was found to be associated with reduced renal function and hypercalcaemia.

Narang *et al.* (1984) studied the effect of vitamin D supplementation on serum calcium levels in humans, with and without tuberculosis. Their diet was supplemented with daily vitamin D doses of 10, 20, 30, 60 and 95 μg/day for 3 months. Thirty healthy males and females ranging in age from 21 to 60 years and without tuberculosis were in one study group. Statistically significant increases in serum calcium were observed in these subjects at vitamin D doses of 60 and 95 μg/day. The mean serum calcium concentration in normal controls following administration of 60 μg/day of vitamin D increased from 2.43 to 2.62 mmol/L, a change that did not indicate hypercalcaemia. However, following 95 μg/day, the mean serum calcium level in normal controls increased from 2.46 to 2.83 mmol/L. No information on the nature of the vitamin D preparation, background vitamin D intake or serum 25(OH)D was given.

The results of Narang and co-workers were not supported by Tjellesen *et al.* (1986). They monitored serum vitamin D metabolites and calcium in 19 healthy premenopausal women during treatment with 100 μg/day of vitamin D_2 or vitamin D_3 for 8 weeks. They found that serum calcium increased significantly by a minute amount of 0.05 mmol/L with 100 μg/day of vitamin D_3. The urinary calcium excretion increased slightly with a mean molar calcium/creatinine ratio of 0.518, which is well below hypercalciuric ratio of 1.0.

In a recent study described above Vieth *et al.* (2001) supplemented healthy volunteers in groups of 33 and 28 healthy individuals with 25 and 100 μg cholecalciferol, respectively, for 1-5 months. In all subjects serum calcium remained within the reference values for serum calcium and no significant change from baseline values were found. Similarly, on a group basis, there was no significant change from baseline in urinary molar calcium/creatinine

ratios. There were more subjects exceeding a ratio of 1.0 in the high dose group than in the low dose group.

3.5.5 Serum 25(OH)D, serum calcium and hypercalcaemia

In patients with rickets, Stamp (1975) demonstrated a parallel increase in serum 25(OH)D and serum calcium during the healing period with ultraviolet light. The treatment did not increase the serum calcium concentration above 2.5 mmol/L, neither did the 25(OH)D concentration increase above 125 nmol/L. Also in patients who had consumed milk excessively fortified with vitamin D, there was a correlation between serum 25(OH)D and serum calcium (Jacobus *et al.*, 1992). Serum samples with calcium concentrations larger than 2.75 (hypercalcaemia) were characterised by serum concentrations of 25(OH)D larger than 200 nmol/L.

Adams and Lee (1997) described four normocalcaemic patients with 25(OH)D concentrations at 177±41 (132-222) nmol/L with hypercalciuria and depressed serum PTH. The intake of vitamin D was in the form of supplements of uncertain magnitude. Upon withdrawal of the supplements the patients became normocalciuric and the 25(OH)D concentration returned to normal (<130 nmol/L).

Better *et al.* (1980) investigated 45 randomly selected Israeli lifeguards who worked at the beach during August-September and compared this group with a control population matched for age and season. Both groups had similar serum calcium levels, but the lifeguards had a significantly lower serum PTH, a higher serum 25(OH)D level (148±105 *vs* 65±25 nmol/L), and a higher urinary calcium excretion. Eleven lifeguards had nephrolithiasis, a significant higher incidence than in the general population. The lifeguards had slightly lower urinary volume than those of controls did.

3.6 Susceptible groups

3.6.1 Infants

The regulation of 1α-hydroxylase and the normal feedback suppression by $1,25(OH)_2D$ on the kidney enzyme seem to work less well in infants than in adults (Stern *et al.*, 1981).

3.6.2 Idiopathic hypercalcaemia of infancy and Williams' syndrome

Idiopathic infantile hypercalcaemia (IIH) and Williams' syndrome are two conditions associated with hypercalcaemia in infancy (Seelig, 1969; McTaggart, 1999; Hockenhull *et al.*, 1999; Rodd and Goodyer, 1999). Both conditions occur sporadically, but inheritance has also been described. Williams' syndrome was described in 1961 by Williams and co-workers and is in more than 90% of the cases caused by a microdeletion on chromosome 7 affecting the elastin gene. The Williams' syndrome is a multisystem developmental syndrome with vascular and connective tissue abnormalities, hypercalcaemia, dysmorphic faces and mental retardation. Levels of $1,25(OH)_2D$ are often elevated and followed by excessive intestinal absorption of calcium.

The "mild" or light variant of IIH is a heterogeneous disorder originally described in the 1950s in England during the period of high-dose vitamin D fortification of milk. Lowering the supplementation dramatically decreased the incidence. The relationship to vitamin D metabolism is unclear. Elevated levels of PTH-related protein, as a cause for the disease, has been found in some cases. Generally, the hypercalcaemia disappears after the first year and

the prognosis is good. Both IIH and Williams' syndrome are treated with a diet low in vitamin D and calcium and there is hypersensitivity towards vitamin D. Hypercalcaemia might develop with vitamin D intakes as small as 5-10 μg/day.

3.6.3 Patients with sarcoidosis, tuberculosis, lymphomas and infants with subcutaneous fat necrosis

The feedback mechanism of $1,25(OH)_2D$ synthesis seems to operate poorly, if at all, in tissues other than that of the renal tubule. In patients with sarcoidosis, $1,25(OH)_2D$ is believed to be synthesised in macrophages, which in these patients have an increased enzyme capacity, or other cells in the granulomas. Also the clearance of $1,25(OH)_2D$ may be decreased as well. Contrary to normal in these patients there is a positive correlation between 25(OH)D within reference levels and $1,25(OH)_2D$ in serum. Even normocalcaemic patients with sarcoidosis have unregulated production of $1,25(OH)_2D$ in response to vitamin D. Also exposure to sunlight may increase the level of active metabolite.

In some lymphomas, typically B-cell lymphomas, there is an increased blood level of $1,25(OH)_2D$, which is probably synthesised by lymphocytes (Narang *et al.*, 1984; Bell, 1998).

Excessive endogenous synthesis of $1,25(OH)_2D$ occurs in children with subcutaneous fat necrosis (Rodd and Goodyer, 1999).

Vitamin D deficiency can mask primary hyperparathyroidism and this could account for the occasional cases of hypercalcaemia observed when large groups of elderly people are given vitamin D supplements (Johnson, 1980).

3.7 Interactions

3.7.1 Vitamin A

Earlier work has provided some evidence that vitamin A might antagonise the actions of vitamin D. Recently this was clearly shown in experiments with rats. Twenty-one day-old male Holtzman rats were fed a rachitogenic diet supplemented with 15.5 ng ergocalciferol (D_2)/day every 3 day and retinyl acetate in doses from 0-8621 ng/day. Increasing the level of retinyl acetate caused a progressive decrease in the total amount of ash in the femur and increase in epiphyseal plate. This antagonistic effect of retinyl acetate was also present even at higher vitamin D_2 dosages. In addition retinyl acetate also inhibited vitamin D_2 action in rats fed a normocalcaemic diet (Rohde *et al.*, 1999). These experiments show that an antagonism takes place at physiological levels of these two vitamins. At the molecular level vitamin D and A share RXR as a common partner for the receptor: the vitamin D receptor heterodimerises with RXR whereas RXR alone or together with RAR function as a mediator of the biological effects of retinoic acids.

3.7.2 Magnesium

Elevation of plasma magnesium increases the secretion of PTH (Rude *et al.*, 1978), which stimulates the synthesis of $1,25(OH)_2D$. Magnesium deficiency in humans, on the other hand, may result in an impaired PTH secretion followed by hypocalcaemia and a reduced serum concentration of $1,25(OH)_2D$. This explains why patients with hypoparathyroidism may be resistant to vitamin D therapy unless magnesium is also given (Fatemi *et al.*, 1991).

3.7.3 Drugs

Ketoconazole, which inhibits the 24-hydroxylase activity, markedly enhances the potency of $1,25(OH)_2D$. Enhanced potency of $1,25(OH)_2D$ is also seen in 24-hydroxylase null mice.

Thiazide drugs, which increase the tubular reabsorption of calcium, would enhance the hypercalcemic effect of a high dose of vitamin D.

Glucocorticoids, phenobarbital and phenytoin antagonise the effect of vitamin D on intestinal calcium absorption. These drugs also protect rats against high doses of vitamin D (Haynes, 1990)

4. DOSE-RESPONSE ASSESSMENT AND DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

4.1 Critical effects

Due to great uncertainty data from animal studies are considered to be inappropriate for identification of critical endpoints and establishment of a NOAEL or a LOAEL.

The principal critical effect of hypervitaminosis D/vitamin D toxicity is hypercalcaemia. It has, however, been reported that patients with hypervitaminosis D (increased level of 25(OH)D >130 nmol/L), hypercalciuria and a depressed PTH status can be normocalcaemic (Adams and Lee, 1997). Thus, hypercalciuria apparently is an earlier phenomenon than hypercalcaemia which could predispose to kidney stone formation.

4.2 Adults

4.2.1 Establishment of a NOAEL on the basis of hypercalcaemia

In the report by Narang *et al.* (1984) a modest hypercalcaemia (level >2.75 mmol/L) was demonstrated at a vitamin D intake of 95 µg/day. The changes in serum calcium following 60 µg vitamin D/day were still within the reference range. There are, however, major drawbacks in this study. There are no data on pre-treatment vitamin D status, no data on sun exposure and no information about the compound given. Neither are there any data on serum 25(OH)D concentrations nor information on the physical status on the healthy participants. Other studies by Tjellesen *et al.* (1986) and Vieth *et al.* (2001) could not confirm the data of Narang *et al.* (1984) as they found no or only a very small increase in serum calcium at an intake of 100 µg vitamin D/day. Increases in the 25(OH)D concentration in serum were seen in both latter studies, but within reference values (<130 nmol/L). The discrepancies between the study of Narang *et al.* (1984) and the latter ones could be vitamin D compound given, vitamin D status before supplementation, body weight, body fat or solar exposure.

4.2.2 Using 25(OH)D in serum for the establishment of a NOAEL

An alternative approach is to use serum 25(OH)D levels as basis for the assessment. Adams and Lee (1997) described hypercalciuria and depressed serum PTH in normocalcaemic patients with plasma 25(OH)D concentrations at 132-222 nmol/L. Interestingly, upon withdrawal of the supplements the patients became normocalciuric and the 25(OH)D

concentration returned to normal (<130 nmol/L). Hypercalciuria was also observed in Israeli lifeguards with a serum 25(OH)D concentration at 148±105 nmol/L (Better *et al.*, 1980).

A NOAEL could also be based on a 25(OH)D concentration in serum of about 150 nmol/L, which is considered the upper reference value, and which has not been reported to be associated with hypercalcaemia or hypercalciuria.

The vitamin D intake associated with exceeding the upper reference value of 25(OH)D in serum would vary greatly in the population. It is, for instance, dependent on the exposure to sunlight and sensitivity to vitamin D. The importance of the chemical form of vitamin D, i.e. vitamin D_2 or D_3 as described above (see 2.5.2) with a lower biological efficiency of vitamin D_2, should be noted. In addition the vehicle used (fat or emulsion) could influence bioavailability. This was shown as early as in 1935 by Stearn and Jeans (cited in Seelig, 1969). Vitamin D from cod liver oil emulsified in milk is about three times as potent as vitamin D given in cod liver oil or propylene glycol. For some individuals an intake of 250 µg vitamin D would not cause an exceed of this value while in others this could occur. The data of Stamp *et al.* (1977) (data taken from figure 1 of Stamp *et al.*, 1977) indicate that the upper reference value of serum 25(OH)D at 150 nmol/L or 200 nmol/L is exceeded by 5% of the population at an approximate vitamin D intakes of about 80 or 100 µg/day, respectively. These levels of 25(OH)D in serum can be considered NOAELs with respect to increased risks of hypercalciuria and hypercalcaemia, respectively. On the other hand, the study by Tjellesen *et al.* (1986) and the recent study of Vieth *et al.* (2001) reported that the upper reference serum concentration of 25(OH)D was not exceeded upon supplementation with 100 µg cholecalciferol (vitamin D_3)/day.

4.2.3 Derivation of an UL for adults

Taking into account all the information in the studies above (particularly information on vitamin D_3 provided by Tjellesen *et al.*, 1986 and Vieth *et al.*, 2001), the risk of hypercalciuria/hypercalcaemia probably starts to increase in some parts of the population at an intake above 100 µg vitamin D/day. The risk of exceeding the upper reference concentration of 25(OH)D in serum will also increase. A dose of 100 µg vitamin D/day and a serum level of 200 nmol 25(OH)D/L are considered a NOAEL. An uncertainty factor of 2 is considered adequate to account for the inter-individual variation. An upper intake level of 50 µg vitamin D/day is considered to offer adequate protection against the risk of hypercalciuria and hypercalcaemia. On this basis the Committee sets an **UL of 50 µg vitamin D/day for adults**[1]**.**

Regarding less frequent intakes of larger doses of vitamin D, this has been discussed above (see section 3.3.2) and no recommendation is given.

No data are available to suggest that other life-stage groups have increased susceptibility to adverse effects of high vitamin D intake. Given the minor impact of circulating vitamin D on calcium levels *in utero* and in breast-fed infants with maternal supplements of 25 and 50 µg vitamin D/day (see section 3.4) there does not seem to be an increased sensitivity during this

[1] It should be noted that the intake of vitamin D via food would add to synthesis caused by exposure to sunlight. Depending on the amount of sunlight the risk of adverse effects at an intake at the upper level would increase. It should also be noted that higher doses of vitamin D might be needed, particular in elderly, to achieve optimal serum levels of 25(OH)D for purpose of optimal mineralisation of the skeleton. Such treatment should be under medical surveillance.

period. Therefore the UL of 50 μg/day should be considered to apply also to pregnant and lactating women.

4.3 Infants

Most of the studies also documenting duration and magnitude of intake in infants regarding adverse effects of vitamin D such as growth retardation and hypercalcaemia are old studies or case reports.

4.3.1 Establishment of LOAEL/NOAEL on the basis of growth and hypercalcaemia

Jeans and Stearns (1938) found retarded linear growth in 9 infants up to one year of age who received 45-112.5 μg vitamin D/day as supplements in comparison with standard growth curves of infants receiving daily supplements at doses of 8.5 μg or less for a minimum of 6 months. The group receiving high vitamin D supplements showed a retarded linear growth and increased rate of growth was seen when the dose of vitamin D was reduced to 10-15 μg/day. The children received both cod liver oil, a cod liver oil concentrate emulsified in cream and viosterol (vitamin D_2). In another study, Fomon *et al.* (1966) compared linear growth in a group of 13 infants daily ingesting 34.5-54.3 μg vitamin D (mean 45 μg) with 11 infants who received 8.8-13.8 μg/day. The infants were enrolled before the age of 9 days and followed at ages 28, 56, 84, 112, 140 and 168 days. No effect on growth was found in this small study. Neither was any hypercalcaemia seen. These studies were performed before vitamin D metabolites in serum could be routinely measured. The authors of the latter study provided some information on the preparation given, Deca-Vi-Sol, but not whether this is vitamin D_2 or D_3.

An epidemic of infantile hypercalcaemia was observed in the UK during 1953 to 1957 when National Dried Milk and other foods including infant food were supplemented with vitamin D. More than 200 cases of hypercalcaemia were reported by June 1955 to 196 consultant paediatricians in the UK. Also other conditions related to vitamin D toxicity increased during this period. In 1957 the vitamin D contents in dried milk, cereals for infants and cod liver oil were reduced: dried milk from 80 to 25 μg/100 g of dry matter, cereals: 8 to 2.5 μg/100 g of dry matter and in cod liver oil from 4 to 2 μg/mL. Following this, the incidence of hypercalcaemia was reduced substantially, but not completely (Seelig, 1969). According to the two surveys conducted by the British Paediatric Association (BPA, 1956 and 1964) there was a reported decline in hypercalcaemia in infants from 7.2 in the 1953-1955 survey to 3 cases per month in the 1960-1961 survey. The estimated total intake of vitamin D in infants for the 75 percentile showed 100 μg/day in the first survey declining to a range of 18 to 34 μg/day in the second survey (BPA, 1956; Bransby *et al.*, 1964). The non-specific early symptoms of hypercalcaemia are failure to thrive, hypotonia and susceptibility to infections would suggest that the diagnosed cases might only be the tip of an iceberg (Seelig, 1969).

Among infants of the age of 6 to 20 months with hypercalcaemia, the intake of vitamin D was estimated to be below 50 μg/day in 50 children (Seelig, 1969). In several American reports of individual cases of infantile hypercalcaemia the vitamin D estimated intake was between 20 and 35 μg/day (cited in Seelig: Bongovanni *et al.*, 1957, N Eng J Med 257: 951; Schwartz, 1957, J Pediat 51: 461; Snyder, 1958, Am J Diseases Children 96: 376; Garcia *et al.*, 1964, N Eng J Med 271: 117; Rashkind *et al.*, 1964, J Pediat 58: 390).

Taken together these early data indicate that at least some infants are sensitive to excessive intake of vitamin D and at risk for hypercalcaemia. It cannot be excluded that some of the cases of infantile hypercalcaemia during this period represent cases of Williams' syndrome or idiopathic infantile hypercalcaemia in addition to those of vitamin D intoxication. With regard to the reports of hypercalcaemia cases in particular could be due to other causes not related to vitamin D. Furthermore, none of these publications had any information on 25(OH)D in serum or any other biomarker of vitamin D status as these studies were performed before such measurements were available. And the magnitudes of the daily vitamin D dosages of the survey data and lack of data on sunlight add to the uncertainties. Thus, it is not possible to exactly define a NOAEL or LOAEL. However, based on these data, it cannot be excluded that an increased risk of vitamin D toxicity and hypercalcaemia might be present at exposures below 50 µg/day. The observations on hypercalcaemia in infants in the UK furthermore indicate that the susceptibility among infants, although less expressed, extends beyond the first year of age.

4.3.2 Establishment of LOAEL/NOAEL on the basis of 25(OH)D in serum and hypercalcaemia

The upper reference level for 25(OH)D for infants is similar to that of adults and the approach used for adults by setting the upper level at an oral dose of vitamin D not associated with exceeding the upper reference level (i.e. 130-150 nmol/L) could in theory be done. A problem is that there are very few data on doses of vitamin D above the recommended intake and corresponding concentrations of 25(OH) in serum.

In a study from Germany, Hövels and co-workers (1983) determined 25(OH)D in serum in infants 12, 18 and 24 months of age. Levels of 25(OH)D were determined in infants 12 and 24 months of age receiving 12.5 (n=58 and n=87) and 25 µg vitamin D_3/day (n=34 and n=15), respectively. It is difficult to evaluate the absolute ranges of 25(OH)D reported in this study since they deviate from those usually reported in other studies and no information on the method of determination and reference values was given in the paper. Thus, this study cannot be used for the establishment of a relationship between vitamin D intake and 25(OH)D or a NOAEL.

In a study from Norway by Markestad (1984), 25(OH)D was measured in eight infants 6 weeks of age in the winter and only receiving a formula containing 10 µg vitamin D_3/L (corresponding to about 8-10 µg/day). The concentrations varied from 60 to 125 (mean 90) nmol/L, showing that this amount of vitamin D was enough to give approximately the reference concentration of 25(OH)D. Thirty-seven 6 and 12 month-old infants who had received vitamin D from cod liver oil (vitamin D_3) or a commercial multivitamin supplement (unknown form of vitamin D) in the following doses: 7.5-10 µg/day (n=23), 5 µg/day (n=9) and 2.5 µg/day (n=5) had in the winter a concentration range of less than 20 to 115 nmol 25(OH)D/L in serum. Twenty-two infants 7 to 18 months of age were studied at the end of the summer. They had not received any vitamin D fortified food or supplements for 4 months, and their serum concentration of 25(OH)D ranged from 30 to 164 (mean 85) nmol/L.

In a study from Finland, Ala-Houhala (1985) supplemented breast-fed infants with 0, 10 and 25 µg vitamin D_2/day for 20 weeks, using 14-17 infants and mothers in each group. In the group where the infants were not given vitamin D, the mothers were given 25 µg/day. Two studies were conducted, one starting in January and one starting in July. No signs of hypercalcaemia were reported. The serum level of 25(OH)D increased rapidly in both groups

of infants supplemented with 10 and 25 μg/day. The levels obtained in the July-December groups were 15-20% higher than those of the groups starting in January. At 20 weeks of age the 25(OH)D level did not show any sign of reaching a steady state level. In the groups supplemented with 10 μg/day the 25(OH)D levels (mean±SEM) were 83±13 and 98±3 nmol/L in the winter and summer groups, respectively. In the groups supplemented with 25 μg/day the 25(OH)D levels (mean±SEM) were 110±13 and 138±7 nmol/L in the winter and summer groups, respectively. (All data were derived from figures 2, 3 and 5 in Ala-Houhala, 1985). The 25(OH)D levels obtained with a supplementation of 10 μg/day seem to agree well with the data of Markestad (1984).

Hesse and co-workers (reported in an abstract, 1993) examined the effects of prophylactic administration of 10 μg vitamin D_3 tablets in 2707 newborn to 15 month-old infants. Infants were breast-fed or given vitamin D-free formula milks. The treatment was started in the second week of life. 25(OH)D increased from 22.6±13.8 to 83.1±36.1 nmol/L from the second week to the third month and to 93.9±36.6 nmol/L between 4-6 months. Elevated 25(OH)D (>130 nmol/L) were found in the 10.1% and 2.6% of the infants in the age of 0-6 months and 6-12 months, respectively. Importantly, elevated serum calcium (>2.8 mmol/L) was observed in 6.4% of the infants in the first 6 months. One of the 2707 infants had rickets. In an extended study (also reported in an abstract, Hesse *et al.*, 1994; Hesse, 1994) it was found that among 3481 infants treated in this way 2.9% of the infants aged 2 weeks to 6 months had 25(OH)D levels above 173 nmol/L (>3 standard deviations) and 0.9% had serum calcium >3.08 mmol/L. On this basis, the authors proposed to reduce the vitamin D supplement to 7.5 and 10 μg D_3/day for the first and second half year of life, respectively, and to the reduce the supplementation of infant formula from 10 to 7 μg D_3/L (Hesse *et al.*, 1993 and 1994; Hesse, 1994). It should be noted that this study was carried out in the eastern part of Germany a few years after the German reunification and that it is possible that the practise of "stossprophylaxis" with vitamin D (see section 3.3.2) had not been discontinued completely. However, no information on this was provided. Because of this and since only limited information is available from the abstract, it is inappropriate to use this study in the derivation of the upper level.

Vervel and co-workers (1997) determined serum 25(OH)D in infants 1 to 4 months of age seen as outpatients The infants received 25 μg vitamin D_2/day in addition to infant formula unfortified (n=23) or fortified with vitamin D (n=41). The 25(OH)D levels in serum was 92.5±28 and 72.8±24.3 nmol/L in the summer and winter, respectively. Those fed vitamin D fortified formula in addition had 99±22 nmol/L in the summer. In a prospective controlled study serum 25(OH)D was determined in healthy neonates born to unsupplemented (n=48) or vitamin D_3 supplemented mothers (n=22) between April and July. The infants were given vitamin D_2 fortified milk, providing either 12.5 or 25 μg vitamin D/day and followed from birth to 3 months of age. At birth, 25(OH)D was 27.4 and 37.8 nmol/L in unsupplemented and supplemented mothers, respectively. After 1 and 3 months there were only marginal differences between the groups receiving 12.5 and 25 μg vitamin D_2 and no differences between the groups from unsupplemented and supplemented mothers, respectively. The values at 3 months were 61.5±12.3 and 61±7 nmol/L, in the groups receiving 12.5 μg vitamin D_2/day, and 75.25±12.5 and 66.25±13 nmol/L, in the groups receiving 25 μg vitamin D_2/day. No values were above 92.5 nmol/L. Serum calcium values at 3 months in the low groups were within 2.42-2.80 mmol/L and in the high groups within 2.46-2.79 mmol/L. The percentage of children with serum calcium above 2.6 mmol/L was not increased in the group receiving 25 μg vitamin D_2/day. The same group (Zeghoud *et al.*, 1997) also examined the increase in 25(OH)D depending on the basal 25(OH)D prior to vitamin D_2 supplementation. The increase

was greatest in the group with the lowest basal level, <15 nmol/L, and only in this group did 25 µg vitamin D_2/day cause an increase in 25(OH)D greater than that produced by 12.5 µg vitamin D_2/day.

4.3.3 Derivation of an UL for infants

Two endpoints are used in the derivation of the UL, namely hypercalcaemia, which can be considered an adverse effect, and the serum concentration of 25(OH)D greater than the upper reference level. The upper reference level of 130-150 nmol/L is well below the threshold of increased risk for hypercalcaemia, which in adults is above 200 nmol/L.

The studies by Fomon *et al.* (1966) recording no effect on growth and serum calcium might indicate a NOAEL of 45 µg/day, however, only a small number of infants (n=13) was studied. The form of vitamin D given in this study is not known. No hypercalcaemia was recorded in the studies of Ala-Houhala (1985) and Vervel and co-workers (1997) supplementing up to 25 µg vitamin D_2/day in addition to breast milk or to formula fortified at least with a level giving 7 µg vitamin D_2/day.

Using the upper reference serum concentration of 25(OH)D (130-150 nmol/L) in deriving an upper level the only data on intake and 25(OH)D concentrations are vitamin D_2 intakes at the upper, middle and the lower range of the PRI. At intakes of 25 µg vitamin D_2/day in addition to breast-feeding the mean level of 25(OH)D (110±13 and 138±7 nmol/L) is close to the upper reference values reported (130-150 nmol/L), particularly when exposed to sunlight in one study (Ala-Houhala, 1985). In another study of non-breastfed infants receiving 25 µg vitamin D_2/day through fortified formula had a slightly lower level of 25(OH)D, i.e. 92.5 nmol and less (Vervel *et al.*, 1997).

Systematic studies that can be used to establish a NOAEL have not been performed on infants receiving more than 10 µg vitamin D_3 in addition to breast milk. However, the present intake of vitamin D_3 in infants were calculated according to current recommendations in Germany on supplement (10 to 12.5 µg vitamin D_3/day to all infants) and use of supplemented infant and follow on formula. It was found that during the first 5 months of life vitamin D_3 intakes could be up to 24 µg/day. The calculated daily intake gradually decreases to up to 16.6 µg/day. The current intake apparently is not associated with any problems of vitamin D excess. It should be noted, however, that no systematic studies have been carried out and that mild hypercalcaemia is associated only with mild and unspecific symptoms such as failure to thrive.

Considering hypercalcaemia the small and old study by Fomon *et al.* (1966) indicated a NOAEL of 45 µg vitamin D/day for infants. However, two more recent and larger well-controlled studies showed that neither in infants receiving 25 µg vitamin D_2/day in addition to breast milk (Ala-Houhala, 1985) nor in infants receiving 32 µg vitamin D_2/day (Vervel *et al.*, 1997) was hypercalcemia observed. In addition, in neither of these studies the resulting serum 25(OH)D concentrations were observed to be above the upper reference level indicating that these are well below the threshold of hypercalcaemia and consequently an uncertainty factor of 1 is considered appropriate. Using the lower value taking into account the higher biological activity and toxicity of vitamin D_3 (see section 2.5.2) and the other information provided above **the Committee sets an UL of 25 µg vitamin D/day for infants 0-24 months of age[2].**

[2] It should be noted that the intake of vitamin D via food would add to synthesis caused by exposure to sunlight. Depending on the amount of sunlight the risks of adverse effects at an intake of the upper level would increase.

4.4 Children and adolescents

4.4.1 Derivation of an UL for the age group 2-17 years

In the age group 2-17 years there are no data on high level intake to support the derivation of an upper level. Finnish 9-15 years old girls were supplemented with vitamin D_2 in the winter season for 3 years (Lehtonen-Veromaa *et al.*, 2002). Before start 14% and 75% of the group had severe (<20 nmol 25(OH)D/L) and moderate (between 25 and 37.5 nmol 25(OH)D/L) hypovitaminosis D at baseline in winter. The first and second year, in addition to a median dietary intake of 4 µg vitamin D/day, they were supplemented from October to January with 10 µg vitamin D_2/day and the third year with 20 µg vitamin D_2/day. The daily supplementation with 120 to 140 µg vitamin D_2/week increased 25(OH)D to 45.5±17.2 nmol/L at 36 months. Sunlight in the summer was apparently more effective raising mean levels to 62 nmol/L. The effect of supplementation or sunlight was greatest in the group with severe hypovitaminosis D. In this study 20 µg ergocalciferol/day was well tolerated, but the nutritional effect of supplementation was rather weak, 11.7 25(OH)D nmol/L for the group taking 80-140 µg ergocalciferol/week. This is probably due to the general low efficiency of vitamin D_2, about 1.7 times less efficient (Trang *et al.*, 1998) than vitamin D_3 as discussed in section 2.5.2. There are no studies on supplementation with high levels of vitamin D_3 in children and adolescents. Apparently, the response to 10 µg vitamin D_2/day on 25(OH)D was much greater in a group of prepubertal Finnish children (Ala-Houhala *et al.*, 1988b). It seems that susceptibility towards vitamin D changes with age. Using a cautious approach taking into consideration a lower weight in children up to 10 years the Committee sets the upper levels as follows:

UL of 25 µg vitamin D/day for children from 2 up to and including 10 years of age[3]

UL of 50 µg/day for adolescents 11-17 years of age[3]

4.5 Summary of upper levels for vitamin D

Age (years)	Tolerable Upper Intake Level (UL) for vitamin D (µg/day)[3]
0-2	25
3-10	25
11-17	50
Adults[+]	50

[+] The UL for adults does also apply to pregnant and lactating women.

[3] It should be noted that the intake of vitamin D via food would add to synthesis caused by exposure to sunlight. Depending on the amount of sunlight the risk of adverse effects at an intake at the upper level would increase. It should also be noted that higher doses of vitamin D might be needed, particular in elderly, to achieve optimal serum levels of 25(OH)D for purpose of optimal mineralisation of the skeleton. Such treatment should be under medical surveillance.

5. RISK CHARACTERISATION

5.1 Adults

2,000 IU UL

An UL of 50 µg vitamin D/day is above 5 times the intake (PRI) recommended by the SCF (1993) of 0-10 µg and 10 µg vitamin D/day for adults below and above 65 years of age, respectively. The mean and 95 percentile intakes without supplements in several European countries are about 10-13 times and about 5 times less than the UL, respectively. In Norway the 95 percentile intake with supplements is about 1.5 times less than the UL. The 97.5 percentile values with supplements are 8.4, 12.7, 14.3 and 22.16 µg/day in Italy, UK, Ireland and Austria, respectively. The 97.5 percentile values without supplements are 8.4, 8.9 and 14.1 µg/day in the UK, The Netherlands and Germany, respectively. These values are well below the UL.

5.2 Infants

400 IU UL

Recent evaluations in several European countries and the USA recommended an intake for infants of 10 µg vitamin D/day to ensure that the population gets enough vitamin D. The recommended intake (PRI) given by the SCF (1993) is 10-25 µg vitamin D/day for infants 6-12 months of age and 10 µg vitamin D/day for infants 12 to 36 months of age. No recommendation is given for the period 0-6 months. Some infants in this age group may reach an intake up to 22-24 µg vitamin D_3/day according to calculations based on current levels of vitamin D_3 in infant formula in Germany (1-2 µg vitamin D_3/100 Kcal) in addition to recommended supplementation (10-12.5 µg vitamin D_3/day). The UL is similar to the upper end of the present PRI of 10-25 µg/day for 6-12 months (SCF, 1993).

It should be kept in mind that hypercalcaemia due to vitamin D excess is uncommon in hospitals. In this matter there is a dilemma because on the one hand it is important to prevent excess intake, on the other hand it is equally important to secure infants an adequate vitamin D intake to avoid serious deficiency problems such as rickets. This disease has been reported in European countries particularly among infants and small children of immigrants (Brunvand and Nordshus, 1996; Uldall *et al.*, 1984).

5.3 Children and adolescents

An UL of 25 µg vitamin D/day is 2.5 times the upper range of recommended intakes for children 2-10 years of 0-10 µg vitamin D/day (SCF, 1993). The mean intake without supplements in European countries is about 5 times less than the UL. In Germany the 97.5 percentile intakes without supplements for the age groups 4-6 years and 7-9 years were 7.5 and 8.7 µg/day, respectively.

1,000 IU UL age 2-10

An UL of 50 µg vitamin D/day is 3.3 times the recommended intake for young individuals (11-17 years) of 0-15 µg vitamin D/day (SCF, 1993). Values without supplements from Germany on 97.5 percentile intakes for the age groups of 10-12 years, 13-14 years and 15-18 years were 9.0, 11.1 and 11.1 µg/day, respectively. These values are about 4-5 times less than the UL.

2,000 IU UL age 11-17

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