Dermatologic Therapy, Vol. 23, 2010, 2–12
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DERMATOLOGIC THERAPY
ISSN 1396-0296

Vitamin D metabolism

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ABSTRACT: Irradiation of human skin with ultraviolet B (280–320 nm) initiates the photochemical conversion of 7-dehydrocholesterol via previtamin D3 to vitamin D3. Vitamin D3 needs for its activation two hydroxylation steps in the liver and kidney. The final product, hormonally active 1α,25-dihydroxyvitamin D3 (calcitriol), arrives via the circulation to its target tissues and acts in a genomic or nongenomic manner. It has been found that human skin irradiated with ultraviolet B also is able to produce calcitriol in substantial amounts. This cutaneous vitamin D3 pathway is unique and, most likely, of considerable relevance for healthy and diseased skin. It is well known that topical application of calcitriol and its analogs can improve hyperproliferative skin diseases. Some studies have convincingly demonstrated that calcitriol and other vitamin D analogs may also be used for the treatment of immunological, inflammatory, and infectious skin diseases. More recently, it has been found that calcitriol or vitamin D analogs have photoprotective effects and can reduce UV-induced deoxyribonucleic acid damage.

KEYWORDS: calcitriol, skin, vitamin D3

Basics of the cutaneous vitamin D3 pathway

The major source of vitamin D3 for most humans is the skin exposed to sunlight or artificial sources of ultraviolet B (UVB) radiation (280–320 nm), which, under usual circumstances, contributes to more than 90% to the serum concentration of vitamin D, the latter being a reflection of cutaneous vitamin D3 synthesis, dietary intake of vitamin D3 and vitamin D2, and, if taken, vitamin D supplement. A photochemical reaction with maximum spectral effectiveness at about 297 nm results in formation of previtamin D3 from 7-dehydrocholesterol (provitamin D3, 7-DHC) in basal and suprabasal layers of the skin (FIG. 1) (1,2).

The effectiveness of UVB on formation of previtamin D3 in the skin is influenced by several UVB-absorbing molecules, i.e., chromophores, in the skin, such as melanin, deoxyribonucleic acid (DNA), ribonucleic acid, proteins, and 7-DHC. 7-DHC absorbs UV radiation between 290 nm and 315 nm, causing it to isomerize, resulting in a bond cleavage between carbons 9 and 10 to form the 9,10-seco-sterol previtamin D3. It is reasonable to assume that the action spectrum for previtamin D3 production in organic solvents and in the skin of rats, chickens, and humans spans wavelengths of between 260 nm and 315 nm (3). Approximately 65% of human cutaneous 7-DHC per unit area is found in the epidermis; the remaining 35% is in the dermis. Determination of the subcellular localization of 7-DHC revealed that most 7-DHC (80%) were in the membrane fraction of epidermal tissue (20% in cytosolic fraction).

Dependent on temperature and time, previtamin D3 undergoes, then, nonenzymatic isomerization to form vitamin D3 (cholecalcioferol, calciol). In contrast to 7-DHC, which is a 5,7-diene, vitamin D3 is a 5,7,19-triene with three conjugated double bonds typical for vitamin D molecules. Experimental evidence indicates that about 50% of the previtamin D3 can isomerize to vitamin D3 within 2.5 hours in the skin. This fact explains the rapid rise in serum levels of vitamin D3 after exposure to UVB

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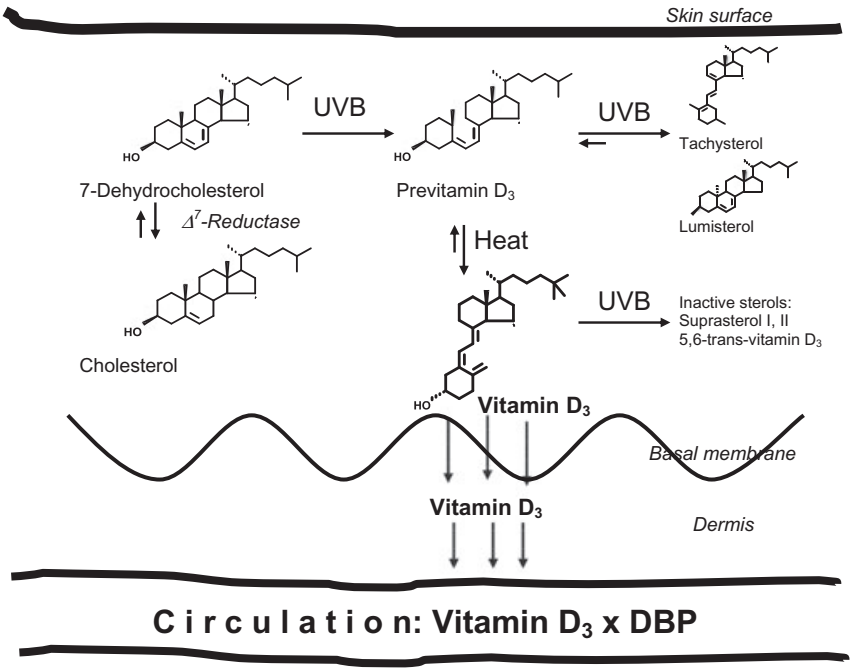

FIG. 1. Photobiology of vitamin D3 in human skin. UVB, ultraviolet B; DBP, vitamin D-binding protein.

radiation. Within 12–24 hours after UVB exposure, the circulating concentrations of vitamin D3 are at their maximum levels. If previtamin D3 is formed in the skin, it can also undergo either photoisomerization to lumisterol, tachysterol, and toxisterols, or is retransformed to 7-DHC. It has been observed that during the first 10 minutes of simulated equatorial solar radiation, about 10–15% of the epidermal 7-DHC in white skin was converted to previtamin D3 without any detectable amounts of lumisterol or tachysterol (4). Another study found that no more than 5% of the 7-DHC in human skin was converted to previtamin D3 (2).

The effects of sun exposure are paradoxical; they include erythema (reddening of the skin after sun exposure) and DNA damage, on one side, and vitamin D3 synthesis, on the other side. The action spectra for previtamin D3 formation, erythema, and formation of cyclobutane pyrimidine dimers from DNA all peak in the UVB range (5). FIG. 2 indicates the similarity of the action spectra for vitamin D3 production and erythema.

Hence, photosynthesis of vitamin D3 cannot be dissociated from acute and chronic photodamage, including photocarcinogenesis (5). In fair-skinned individuals, maximum possible vitamin D3 synthesis occurs within a few minutes of summer sun exposure. Maximum vitamin D3 synthesis in all individuals is generated at suberythemogenic UV doses, and longer exposures add nothing to vitamin D stores despite increasing DNA damage

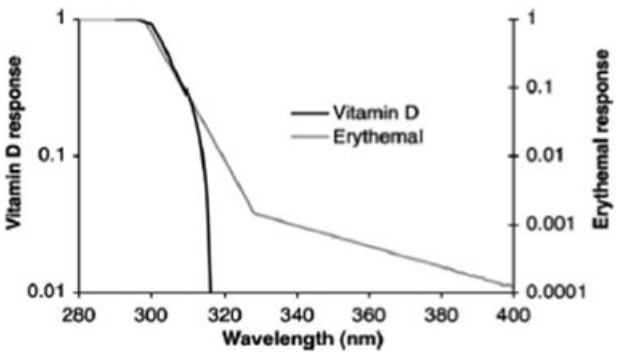

FIG. 2. Action spectra for vitamin D3 production and erythema (6,7).

in a linear fashion. FIG. 2 shows the wavelength dependency of UV for the development of erythema, with the UVB (280–320 nm) wavelengths causing the strongest response. However, there is an impact on erythema due also to the UVA (320–400 nm) wavelengths. To date, the most cited vitamin D3 action spectrum is that of MacLaughlin et al. (2), which was obtained by irradiating neonatal foreskin with UV. As shown in FIG. 2, vitamin D3 synthesis is strictly confined to the UVB region and cannot occur in the UVA region (>320 nm). It should be noted, however, that there is at least a statistical likelihood of previtamin D3 formation at UVA wavelengths. In sunlight and using the definition of the CIE (International Commission on Illumination) of UVB (280–315 nm) (7), any UVA

production of previtamin D3 is of the order of 3–4% of the total production. If one takes the looser definition of UVB favored by dermatologists (280–320 nm), then there is only <1% previtamin D3 formation at UVA wavelengths (3).

Limiting factors of the cutaneous vitamin D3 synthesis

Major sources of vitamin D for most humans are casual exposure of the skin to solar UVB (280–320 nm) radiation and from dietary intake. The effectiveness of cutaneous synthesis of vitamin D3 is determined by several factors (8): (i) the content of 7-DHC in the skin; (ii) the cutaneous concentration of 7-DHC is mainly regulated by the activity of the 7-DHC-Δ^7-reductase, which catalyzes the conversion of 7-DHC to cholesterol and vice versa (FIG. 1) (9); (iii) the energy of photons that depends on the wavelength of the UVB radiation (8); (iv) both the solar zenith angle (which is a function of latitude and season) and time of day (10); (v) skin pigmentation (11) and use of sunscreens (12,13), which considerably suppress photolysis of 7-DHC; (vi) temperature, which regulates the conversion of previtamin D3 to vitamin D3; (vii) exposure doses of UVB because maximal vitamin D synthesis occurs following suberythemogenic UVB exposure, hence higher doses would cause conversion of previtamin D3 to inactive isomers, such as lumisterol, tachysterol, toxisterols, and 7-DHC (4), and of vitamin D3 to suprasterols and 5,6-trans-vitamin D3 (14,15); and (vii) age, because there is an inverse relation between the concentration of 7-DHC in the epidermis with age (16).

Vitamin D metabolism

The two forms of vitamin D (D3 and D2) are biologically inactive; they require activation in the liver and kidney. After binding to carrier proteins, in particular, vitamin D-binding protein (DBP), vitamin D is transported to the liver where it is enzymatically hydroxylated to 25-hydroxyvitamin D [calcidiol, 25(OH)D]. Hydroxylation is catalyzed by a microsomal cytochrome P450 enzyme *CYP2R1* and/or the mitochondrial cytochrome P450 *CYP27A1*; neither is subject to tight regulation. Recently, it has been found that several other cytochrome P450 mixed function oxidases (*CYP2C11*, *CYP3A4*, *CYP2D25*, and *CYP2J3*) exhibit vitamin D 25-hydroxylase activities (17,18). 25(OH)D quickly enters the circulation, where it has a half-life of about 15 days (19). The normal circulating levels of 25(OH)D in the blood are between 25 nmol/L–200 nmol/L. Numerous studies have demonstrated the positive correlation between whole body exposure to solar (or solar-simulated) radiation and rise of circulating 25(OH)D3. It has been shown, for example, that irradiation with a suntanning lamp (MedSun, Wolff Systems Technology, Atlanta, USA) three times a week for 7 weeks (cumulative irradiance: four minimal erythema dose [MED]) resulted in a 50% increase of 25(OH)D3 after 1 week that continued to increase for 5 weeks before reaching a plateau at about 150% above baseline values (20). Currently, serum levels of about 30 ng/mL (75 nmol/L) are considered by many investigators as optimal for health.

25-Hydroxyvitamin D, bound to DBP, is then transported to the kidneys and is finally hydroxylated by *CYP27B1* (25-hydroxyvitamin D-1α-hydroxylase; 1αOHase) at C1α position to hormonally active 1α,25-dihydroxyvitamin D. The 1α-hydroxylation of 25(OH)D to calcitriol is tightly regulated by the parathormone; other regulators are calcium, phosphate, calcitonin, fibroblast growth factor 23, and 1α,25$(OH)_2$D3 itself. Calcitriol has biologic effects in the kidneys but is also transported by DBP to other vitamin D receptor (VDR)-positive target tissues (mainly bone, intestine, and parathyroid gland) to act in a genomic or nongenomic manner (FIG. 2). Regulation of gene expression by calcitriol is mediated by VDR and takes place within hours. By contrast, nongenomic responses of calcitriol are probably mediated by a specific membrane-bound VDR and occur within seconds to minutes. Nongenomic effect of calcitriol include rapid changes in phosphoinositide metabolism, increases in intracellular calcium levels, stimulation of intestinal calcium transport and phosphate fluxes, elevation in cyclic guanosine monophosphate (cGMP) levels, and activation of protein kinase C. The serum levels of calcitriol range from 75 pmol/L to 200 pmol/L; calcitriol has a serum half-life of 10–24 hours (21).

Extrarenal synthesis of calcitriol

There is substantial evidence for additional extrarenal sites of calcitriol synthesis (FIG. 3).

In vitro, many nonrenal tissues, including bone, placenta, prostate, keratinocytes, macrophages, T-lymphocytes, dendritic cells, and several cancer cells (e.g., those from lung, prostate, and skin) can enzymatically convert 25(OH)D to 1α,25(OH)2D (22–25). Several cell types, including epidermal keratinocytes, macrophages, prostate epithelial

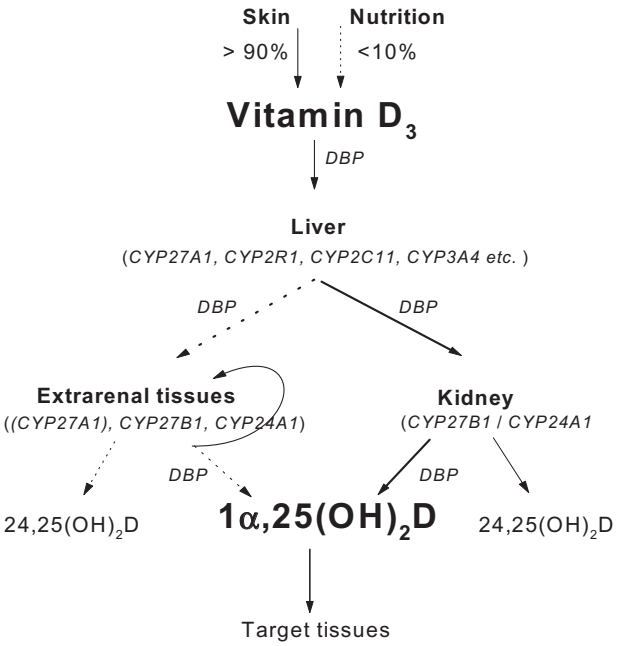

FIG. 3. Renal and extrarenal calcitriol synthesis. DBP, vitamin D-binding protein.

cells, and osteoblasts, express both 25-hydroxylase and 1α-hydroxylase activity, which enables them to metabolize vitamin D3 to 1α,25(OH)2D3 (24,26–29). It has been discovered that human keratinocytes exhibit an autonomous vitamin D3 pathway not only in vitro (30–32), but also in vivo (33) (FIG. 4). However, it should be noted that cutaneous metabolism of circulating 25(OH)D to 1α,25(OH)2D is thought not to play a significant role in vivo because the amount of free 25(OH)D, which has to penetrate the cell membrane of epidermal keratinocytes, is too small to induce formation of sufficient amounts of 1α,25(OH)2D. In particular, 25(OH)D3 is very tightly bound to DBP ($Kd = 5 \times 10^{-8}$ M) in the serum (34). Because of this tight binding and the high plasma concentration of DBP (0.3–0.5 mg/mL), virtually all 25(OH)D3 molecules in the circulation are present in a complex with DBP, and only approximately 0.03% (equivalent to 12.4 ± 4.5 pmol/L) of this metabolite is found in free form (35). Moreover, the epidermis is not vascularized, which further limits the passage of 25(OH)D from blood to epidermal keratinocytes. Keratinocytes also possess vitamin D catabolic pathways. A five-step inactivation pathway from calcitriol to calcitroic acid in epidermal keratinocytes is attributed to the multifunctional 25-hydroxyvitamin D3-24-hydroxylase (*CYP24A1*), which is transcriptionally induced by the action of calcitriol in a very sensitive manner (36). The physiological importance of a second catabolic pathway, which results in the conversion of 1α,25(OH)2D3 to the A-ring diastomer 1α,25(OH) 2D-3epi-D3, is less clear (37).

In vitro investigations have shown that dermal fibroblasts express one of the potential 25-hydroxylases (*CYP27A1*), but not the 1α-hydroxylase (*CYP27B1*) (FIG. 4). Therefore, fibroblasts might play an important role in supplying calcitriol precursors [vitamin D3 and 25(OH)D3] for keratinocytes and, possibly, for the serum (38).

In recent studies with an in vitro system of reconstituted cytochrome P450 side-chain cleavage system (P450scc), 7-DHC and vitamin D3 were found to serve as alternative substrates for P450scc (39). It has been demonstrated that P450scc located in mitochondria from skin cells and other tissues can transform 7-DHC to 7-dehydropregnenolone (7-DHP) (40). 7-DHP may serve as a substrate for further conversions into hydroxy derivatives through steroidogenic enzymes. In the skin, 5,7-steroidal dienes (7-DHP and its hydroxy derivatives) may undergo UVB-induced isomerization to vitamin D3-like derivatives. This novel pathway can generate a variety of compounds depending on local steroidogenic activity and exposure to UVB. The physiological importance of this pathway remains, however, to be clarified. In addition, photosynthesized vitamin D3 can also be sequentially hydroxylated in the epidermis by a monooxygenase encoded by *CYP11A1* to 20,22-dihydroxyvitamin D3 and other, as yet uncharacterized, trihydroxylated vitamin D3 metabolites (39,40).

Cutaneous production of calcitriol may exert autocrine effects on keratinocytes as well as paracrine effects on neighboring cells. This hormone may regulate growth, differentiation, apoptosis, and other biological processes. Skin cells (keratinocytes, fibroblasts, and other cells) express VDR, an absolute prerequisite for regulation of genomic effects of calcitriol and other synthetic vitamin D analogs. There is a multitude of genes in primary human keratinocytes and squamous carcinoma cell lines regulated by calcitriol and its low calcemic analogs (41–43). Notable among these genes are those responsible for regulation of cell growth, differentiation, inflammation, and other processes. Regulation of genes associated with growth and differentiation of keratinocytes argues, in particular, for a link of therapeutic effect of UVB radiation in the treatment of psoriasis with the cutaneous vitamin D3 pathway.

Interestingly, Su et al. (44) have previously demonstrated that free concentrations of calcitriol as low as 10^{-12} M increased involucrin and trans-

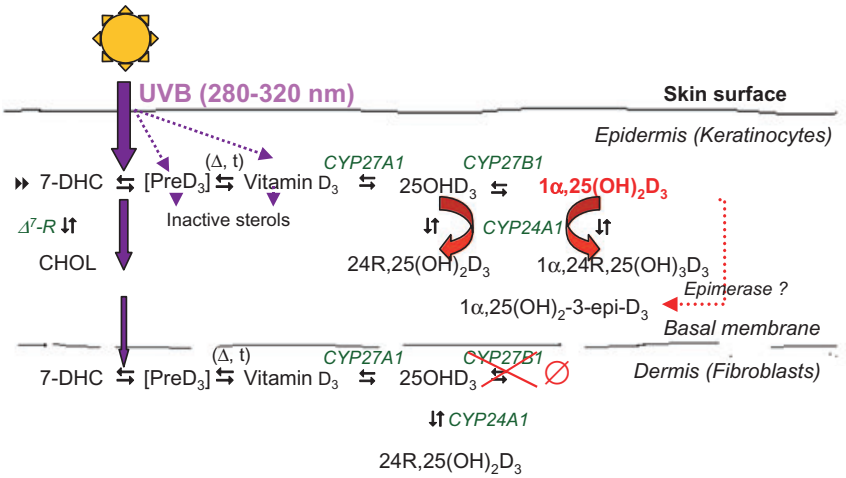

FIG. 4. Metabolism of vitamin D3 in human skin. UVB, ultraviolet B; DBP, vitamin D-binding protein; CHOL, cholesterol.

glutaminase messenger ribonucleic acid levels in keratinocytes in vitro. This sensitive effect of calcitriol might primarily contribute to increased differentiation of keratinocytes in vitro and in vivo. It should also be mentioned that selected transcriptional activity of VDR may occur in keratinocytes irrespective of the presence of the 1α,25(OH)2D3 ligand (45). It has been shown that VDR has the ability to activate the 24-hydroxylase (*CYP24A1*) promoter independently from the presence of 1α,25(OH)2D3 in primary keratinocytes (45). Therefore, a more detailed elucidation of the pathways leading to 1α,25(OH)2D3-independent VDR transcription would be of uttermost interest.

Genomic effects of calcitriol

Upon binding to calcitriol, the VDR is phosphorylated and recruits one of the three 9-cis-retinoid X receptors. Regulation of gene expression is then dependent on the ability of these heterodimers to build co-regulatory protein complexes including the steroid receptor coactivators and the VDR interacting protein. These complexes bind to specific genomic sequences in the promoter region named vitamin D response elements. The VDR not only directly upregulates gene transcription (e.g., *CYP24A1* and genes encoding for cathelicidin) but also directly downregulates the transcription of several genes such as those encoding parathyroid hormone (PTH) or parathyroid hormone-related peptides (PTHrP).

Nongenomic effects of calcitriol

In addition to its genomic effects, calcitriol, like other hormones, mediates these effects through rapid nongenomic actions. Calcitriol activates a variety of signal transduction systems including Ca^{2+} influx; release of Ca^{2+} from intracellular stores; modulation of adenylate cyclase, phospholipase C, and protein kinases C and D; as well as mitogen-activated protein (MAP) and rapidly growing fibrosarcoma (Raf) kinase pathways. These activities have been found in many cells, including keratinocytes, enterocytes (intestinal absorptive cells), muscle cells, osteoblasts, and chondrocytes. VDR seems to be necessary for some of these nongenomic transduction processes; however, another protein named 1α,25-dihydroxy-membrane associated rapid response steroid binding (MARRS) is also seemingly involved in these rapid nongenomic actions.

Exogeneous sources of vitamin D

Vitamin D comprises two closely related substances of nutritional importance: vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Vitamin D3 is formed from its precursor 7-DHC, which is found in ample amounts in the skin of humans and animals. Vitamin D2 is formed by UV radiation from its precursor ergosterol, which is present in plants, yeast, and fungi. However, plants are a poor source of vitamin D2. The two forms of vitamin D only differ by the side chain to the sterol skeleton. In 1950, the World Health Organization (WHO) stated that 1 IU of vitamin D should be equivalent to 25 ng crystalline vitamin D3, and no distinction was made between vitamin D3 and vitamin D2 (46). Of note, orally administered vitamin D3 increases the serum vitamin D status more efficiently (by a factor of 1.7) than vitamin D2 when given in equimolar amounts over 14 days to

healthy volunteers (47). Some studies have shown that vitamin D2 supplementation can suppress endogenously formed 25(OH)D3 and also 1α,25(OH)2D3 (48–50). Therefore, the assumption that vitamins D2 and D3 have equal nutritional value is probably incorrect and should be reconsidered (51). In fact, in recent years, there has been a trend of replacing vitamin D2 with vitamin D3 as the form added to food or given as supplements. Therefore, care should be taken to specify the type of vitamin D used for nutritional studies (47,51).

Dietary sources of vitamin D

Only a few foods naturally contain appreciable amounts of vitamin D3 that have an impact on dietary intake: fish liver, fish liver oils, fatty fish, and egg yolks. Oily fish such as salmon, mackerel, and bluefish are excellent sources of vitamin D3. Interestingly, investigations have shown that farmed salmon, the most widely consumed fish in the United States, contained about one quarter of the vitamin D3 found in wild-caught salmon from Alaska (52). Some farmed salmon even had vitamin D2 as verified by liquid chromatography coupled with tandem mass spectrometry. Altogether, there is the necessity of reevaluation of the vitamin D content in all fish and other foods that have been traditionally recommended as good sources of naturally occurring vitamin D (52).

Some countries practice fortification of certain foods with vitamin D, most often milk, margarine, and/or butter. The mean intakes of vitamin D in different studies vary with age group, food and supplementation habits, and gender.

Vitamin D supplements

Numerous vitamin D supplements in different dosages are widely and inexpensively available in most countries.

Recommendations for vitamin D intake

WHO

Most countries have their own recommendations for vitamin D intake, recognizing that there may be insufficient sun exposure in larger or smaller groups of the population. The WHO published a report on diet, nutrition, and the prevention of chronic diseases in 2003 (53). The osteoporosis section suggested that in countries with a high fracture incidence, low calcium intake (<400–500 mg/day) was associated with increased risk in older individuals. It was suggested that an increase in dietary intake of vitamin D and calcium in this group could reduce fracture risk. Currently, the WHO guidelines indicate that if sunshine exposure was limited, a vitamin D intake of 5–10 μg (200–400 IU) daily was recommended (53).

Europe

Most European countries have their own recommendations for vitamin D intake (54). A sufficient vitamin D intake is recommended in most countries "from the cradle to the grave." Because vitamin D is a fat-soluble vitamin, 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 (≈25 IU – equivalent to 625 ng vitamin D per liter of milk) from women living in industrialized countries is not considered to be sufficient to maintain adequate vitamin D status in the child.

Thus, many countries recommend 10 μg vitamin D per day (400 IU/day) to infants from 4 weeks onwards. The same amount is recommended for pregnant and lactating women. The current recommended daily intake of vitamin D in most European countries is 5 μg/day (200 IU/day) for adults and 10 μg /day (400 IU/day) for those older than 60–65 years. Several European countries often have more detailed recommendations than the general ones, and the recommended values vary somewhat. The Population Reference Intake recommended by the European Community Scientific Committee for Food (SCF) (55) for daily vitamin D intake 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; over 65 years, 10 μg; pregnancy, 10 μg; and during lactation, 10 μg.

However, safety is always an important factor when formulating recommendations for nutrient intake. According to the Food and Nutrition Board (FNB) and using similar methodology, the European Commission SCF also identified a vitamin D3 upper (intake) limit of 50 μg per day (2000 IU/day). The SCF selected 100 μg from the results of the clinical trial of Vieth et al. (56) as the no observed adverse effect level (NOAEL) and selected an uncertainty factor of 2 to calculate the 50-μg UL. Tolerable ULs for vitamin D were set in 2002 by the SCF for special groups of the population (newborns, infants, children, adolescents, adults, as well as pregnant and lactating women) (Table 1).

Table 1. Age-dependent tolerable upper intake limit (UL) for vitamin D (μg/day)

Years	UL
0–2	25 μg (1000 IU)
3–10	25 μg (1000 IU)
11–17	50 μg (2000 IU)
Adults over 50[a]	50 μg (2000 IU)

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

Unfortunately, the SCF has neglected to define the biochemical form of vitamin D, which is selected for application. It is not clear whether the SCF means vitamin D3 or vitamin D2. It should be noted that a 50,000-IU dosage of vitamin D2 is considered to be equivalent, in terms of the conversion rate to 25(OH)D, to no more than 15,000 IU of vitamin D3 and perhaps closer to only 5000 IU. In a study by Armas et al., single doses of vitamins D2 and D3 led to equivalent increases in serum 25(OH)D levels in the initial 3 days. 25(OH)D continued to rise in the vitamin D3-treated individuals, peaked at Day 14, and serum levels remained sustained over 28 days. In contrast, the vitamin D2-treated patients had a rapid decline in serum levels after Day 3 to no change in baseline at Day 14 (57). In other words, the currently tolerable upper intake level of 2000 IU/day for vitamin D3 should not be applied to vitamin D2. However, it has recently been reported that vitamin D2 is as effective as vitamin D3 in maintaining concentrations of 25(OH)D (58).

The recommended daily intake of vitamin D in *Finland*, *Germany*, and *the Netherlands* is 5–10 μg/day (59–61), and 15 μg of vitamin D per day for elderly subjects with insufficient vitamin D3 synthesis in the Netherlands (61). In Germany, the mean vitamin D intake is 3 μg/day in females and 4 μg/day in males (62). Other populations in Europe (Austria, UK, Italy, and Ireland) have a similar recommended vitamin D intake between 3 μg/day and 6 μg/day.

Public health policy in the UK related to nutrition and bone health has been shaped by reports from the Department of Health (DH), Food Standards Agency, and the WHO. Dietary Reference Values for a number of nutrients were published in 1991 by the DH Committee on Medical Aspects of Food and Nutrition Policy. The Dietary Reference Values for vitamin D were based on the dietary amount required to ensure that the serum level of 25(OH)D in winter was above 20 nmol/L (8 ng/mL), as vitamin D deficiency as osteomalacia only occurs in individuals with lower circulating concentrations. The subsequent DH report on nutrition and bone health in 1998 not only concentrated particularly on calcium and vitamin D but also briefly addressed the effect of body weight, alcohol, and other nutrients. However, no changes to the Reference Nutrient Intake (RNI) were made. No RNI was set for children above the age of 3 years or adults below the age of 65 years, unless they were considered at risk of vitamin D deficiency. Individuals whose exposed skin is covered on a regular basis by clothing, those who are house bound, or those having increased skin pigmentation are among the at-risk populations considered when a daily RNI of 10 μg (400 IU) was established. As the mean intake of vitamin D from food sources in adults in the UK ranges from 2.0 μg to 4.0 μg (80–160 IU) daily, most individuals are at risk of developing vitamin D deficiency and will require supplementation.

The *Norwegian National Council on Nutrition and Physical Activity* has recommended daily consumption of cod liver oil supplements, partly because of the suspected vulnerability to vitamin D deficiency in the Norwegian population in relation to low intake in the diet and limited exposure to sunshine, which is the main source of vitamin D3 (63). One dose of cod liver oil supplement (5 mL) contains 500 μg vitamin A, 10 μg vitamin D, and 10 mg vitamin E, as well as 1,2 g n-3 fatty acids (64). Norwegians have a high consumption of vitamin D-rich fatty fish and usually consume cod liver oil during their whole life span (65). This may explain the relatively high levels of serum 25(OH)D in elderly Norwegians during wintertime. Because of varying recommendations in the various countries in Europe, the European Union is supporting a project toward a strategy for optimal vitamin D fortification named OPTIFORD (66).

North America (United States and Canada)

Current recommendations for the Dietary Reference Intake of Vitamin D in the United States by the Institute of Medicine are 5 μg/day (200 IU/day) for newborns, children, and adults aged between 1 month and 50 years; 10 μg/day (400 IU/day) for adults aged between 51 years and 70 years; and 15 μg/day (600 IU/day) for individuals >70 years (67). These guidelines are currently undergoing review. The 2005 Dietary Guidelines for Americans, published by the US DH and Human Services and the US Department of Agriculture, recommend that older adults and other at-risk populations consume 25 μg (1000 IU) of vitamin D daily (68). The American Academy of Dermatology has also

recommended that adults should take 1000 IU of vitamin D3. The American Academy of Pediatrics has recommended that infants, children, and adolescents up to the age of 18 years should take 400 IU of vitamin D daily (69,70). A combination of dietary intake and vitamin D supplementation may be needed to achieve 1000 IU daily.

The US FNB also evaluated the potential for high intakes of vitamin D to produce adverse effects and set a safe tolerable upper intake level of 50 μg (2000 IU) for vitamin D3. The FNB selected 60 μg (2400 IU) as the NOAEL on the basis of evidence obtained from the clinical trial of Narang et al. (71) and selected an uncertainty factor of 1.2 to calculate the 50-μg UL. Recent studies suggest that an oral vitamin D intake up to 100 μg/day is safe in the adult population (56).

In Canada, the Canadian Cancer Society has also recommended a daily intake of 1000 IU of vitamin D (72).

Australia/New Zealand

The current Australian guidelines for recommended vitamin D intake for different age groups are 200 IU/day from birth to 50 years of age, 400 IU/day for people aged 51–70 years, and 600 IU/day for those over 71 years (73).

Special groups

Pregnant and lactating women

Some studies have shown that vitamin D metabolism is changed in pregnant but not in lactating women. Pregnancy is characterized by an increase in the maternal serum level of 1α,25(OH)2D3 (74) because of a putative placental synthesis of this hormone (75). However, the physiological role of the elevated circulating 1α,25(OH)2D3 is not clear. It seems, however, that changes in vitamin D metabolism of pregnant woman do not have a big influence on the maternal vitamin D requirement. However, it is very clear that transfer of vitamin D from mother to fetus is important for the neonate's growth rate and bone development, and probably for other biological processes. In contrast, two studies have failed to indicate any change in serum levels of vitamin D metabolites during lactation (76,77). Increased calcium requirements are mainly regulated by the PTH-related peptide (76,78). The vitamin D content of human milk is relatively low and ranges from 25 IU/L to 40 IU/L (0.6–1 μg/L) maximally (79). Because human milk is a poor source of vitamin D, rickets are still found, but these are almost exclusively in breast-fed infants deprived of sunlight exposure (80,81). There is little evidence that increasing calcium or vitamin D supplementation to lactating mothers results in an increased transfer of calcium or vitamin D in milk (76). Therefore, it seems that there is little purpose in recommending additional vitamin D for lactating women. Vitamin D3 supplementation (400 IU/day) of breast-fed infants, as recommended by the American Academy of Pediatrics, should be practiced (70).

Newborns

Infants have a relative high need of vitamin D because of their high rate of skeletal growth. At birth, infants have acquired in utero the vitamin D reserves that must carry them through the first months of the life. It has been found that 64% of French neonates have serum levels below 30 nmol/L (<12 ng/mL), which corresponds/complies to a severe vitamin D deficiency (82). As stated previously, breast-fed infants are particularly at risk because of the low concentrations of vitamin D in human milk (79). Additionally, the situation worsens by restriction in exposure to sunlight for seasonal, latitudinal, cultural, or social reasons. Infants born in the autumn months at extreme latitudes are particularly at risk because they spend the first months of life indoors and therefore have scarce opportunity to synthesize vitamin D3 in their skin during this period. Accordingly, sporadic cases of rickets are still being reported in many northern cities but are almost always in infants fed with human milk (80–84). All infant formulas sold in the United States actually have at least 400 IU/L of vitamin D (85). Thus, if an infant is ingesting at least 500 mL per day of formula (vitamin D concentration: 400 IU/L), he or she will receive a vitamin D intake of 200 IU per day.

Elderly people

Several studies have demonstrated an age-related decline in many metabolic steps of the vitamin D pathway (86), including the rate of synthesis in the skin, the rate of hydroxylation, and the response of target tissues (e.g., bone) (87). In contrast, a recent study (88) has concluded that intestinal absorption of vitamin D is not decreasing with age, as earlier thought (50). Vitamin D deficiency is then characterized by low serum levels of 25(OH)D coupled with elevations in plasma PTH and alkaline phosphatase (89).

Meta-analysis of randomized clinical trials for hip and nonvertebral fractures showed that vitamin D intake of 700–800 IU/day, but not 400 IU/day, was associated with protection against these fractures (90). In another study, it was found that improving calcium and vitamin D nutritional status substantially reduces all cancer risks in postmenopausal women (91). Other groups have found contradictory results: Calcium plus vitamin D did not prevent fractures or colorectal cancer in postmenopausal women, although it should be noted that only 400 IU/day of vitamin D3 supplement were given to the participants (92,93).

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