

3. Vitamin D

3.1 Role of vitamin D in human metabolic processes

Vitamin D is required to maintain normal blood levels of calcium and phosphate, which are in turn needed for the normal mineralization of bone, muscle contraction, nerve conduction, and general cellular function in all cells of the body. Vitamin D achieves this after its conversion to the active form 1,25-dihydroxyvitamin D [1,25-(OH)₂D], or calcitriol. This active form regulates the transcription of a number of vitamin D-dependent genes which code for calcium-transporting proteins and bone matrix proteins.

Vitamin D also modulates the transcription of cell cycle proteins, which decrease cell proliferation and increase cell differentiation of a number of specialized cells of the body (e.g. osteoclastic precursors, enterocytes, keratinocytes). This property may explain the actions of vitamin D in bone resorption, intestinal calcium transport, and skin. Vitamin D also possesses immunomodulatory properties that may alter responses to infections in vivo. These cell differentiating and immunomodulatory properties underlie the reason why vitamin D derivatives are now used successfully in the treatment of psoriasis and other skin disorders.

3.1.1 Overview of vitamin D metabolism

Vitamin D, a seco-steroid, can either be made in the skin from a cholesterol-like precursor (7-dehydrocholesterol) by exposure to sunlight or can be provided pre-formed in the diet (1). The version made in the skin is referred to as vitamin D₃ whereas the dietary form can be vitamin D₃ or a closely-related molecule of plant origin known as vitamin D₂. Because vitamin D can be made in the skin, it should not strictly be called a vitamin, and some nutritional texts refer to the substance as a prohormone and to the two forms as colecalciferol (D₃) and ergocalciferol (D₂).

From a nutritional perspective, the two forms are metabolized similarly in humans, are equal in potency, and can be considered equivalent. It is now firmly established that vitamin D₃ is metabolized first in the liver to 25-hydroxyvitamin D (calcidiol) (2) and subsequently in the kidneys to

1,25-(OH)₂D (calcitriol) (3) to produce a biologically active hormone. The 1,25-(OH)₂D compound, like all vitamin D metabolites, is present in the blood complexed to the vitamin D-binding protein, a specific α -globulin. Calcitriol is believed to act on target cells in a similar way to a steroid hormone. Free hormone crosses the plasma membrane and interacts with a specific nuclear receptor known as the vitamin D receptor, a DNA-binding, zinc-finger protein with a relative molecular mass of 55 000 (4). This ligand-receptor complex binds to a specific vitamin D-responsive element and, with associated transcription factors (e.g. retinoid X receptor), enhances transcription of mRNAs which code for calcium-transporting proteins, bone matrix proteins, or cell cycle-regulating proteins (5). As a result of these processes, 1,25-(OH)₂D stimulates intestinal absorption of calcium and phosphate and mobilizes calcium and phosphate by stimulating bone resorption (6). These functions serve the common purpose of restoring blood levels of calcium and phosphate to normal when concentrations of the two ions are low.

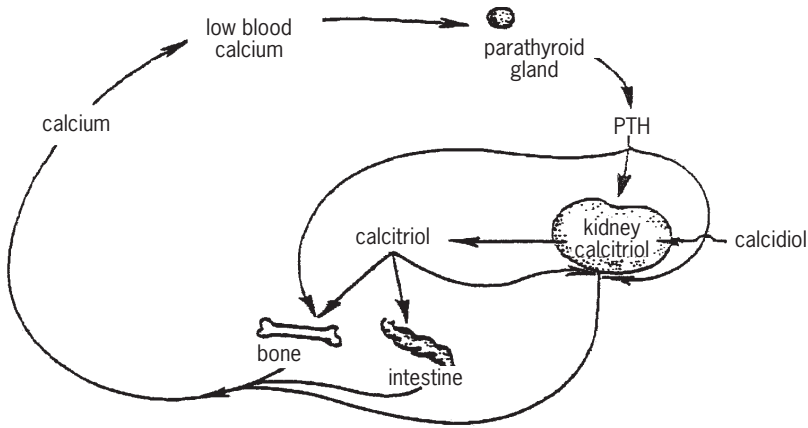
Lately, interest has focused on other cellular actions of calcitriol. With the discovery of 1,25-(OH)₂D receptors in many classically non-target tissues such as brain, various bone marrow-derived cells, skin, and thymus (7), the view has been expressed that 1,25-(OH)₂D induces fusion and differentiation of macrophages (8, 9). This effect has been widely interpreted to mean that the natural role of 1,25-(OH)₂D is to induce osteoclastogenesis from colony forming units (i.e. granulatory monocytes in the bone marrow). Calcitriol also suppresses interleukin-2 production in activated T-lymphocytes (10, 11), an effect which suggests the hormone might play a role in immunomodulation *in vivo*. Other tissues (e.g. skin) are directly affected by exogenous administration of vitamin D, though the physiologic significance of these effects is poorly understood. The pharmacologic effects of 1,25-(OH)₂D are profound and have resulted in the development of vitamin D analogues, which are approved for use in hyperproliferative conditions such as psoriasis (12).

Clinical assays measure 1,25-(OH)₂D₂ and 1,25-(OH)₂D₃, collectively called 1,25-(OH)₂D. Similarly, calcidiol is measured as 25-OH-D but it is a mixture of 25-OH-D₂ and 25-OH-D₃. For the purposes of this document, 1,25-(OH)₂D and 25-OH-D will be used to refer to calcitriol and calcidiol, respectively.

3.1.2 Calcium homeostasis

In calcium homeostasis, 1,25-(OH)₂D works in conjunction with parathyroid hormone (PTH) to produce its beneficial effects on the plasma levels of ionized calcium and phosphate (5, 13). The physiologic loop (Figure 3.1) starts with the calcium receptor of the parathyroid gland (14). When the level of

FIGURE 3.1
Calcium homeostasis



Source: adapted, with permission from the authors and publisher, from reference (13).

ionized calcium in plasma falls, PTH is secreted by the parathyroid gland and stimulates the tightly regulated renal enzyme 25-OH-D-1- α -hydroxylase to make more 1,25-(OH)₂D from the large circulating pool of 25-OH-D. The resulting increase in 1,25-(OH)₂D (with the rise in PTH) causes an increase in calcium transport within the intestine, bone, and kidney. All these events raise plasma calcium levels back to normal, which in turn is sensed by the calcium receptor of the parathyroid gland. The further secretion of PTH is turned off not only by the feedback action of calcium, but also by a short feedback loop involving 1,25-(OH)₂D directly suppressing PTH synthesis in the parathyroid gland (not shown in Figure 3.1).

Although this model oversimplifies the events involved in calcium homeostasis, it clearly demonstrates that sufficient 25-OH-D must be available to provide adequate 1,25-(OH)₂D synthesis and hence an adequate level of plasma calcium; and similarly that vitamin D deficiency will result in inadequate 25-OH-D and 1,25-(OH)₂D synthesis, inadequate calcium homeostasis, and a constantly elevated PTH level (i.e. secondary hyperparathyroidism).

It becomes evident from this method of presentation of the role of vitamin D that the nutritionist can focus on the plasma levels of 25-OH-D and PTH to gain an insight into vitamin D status. Not shown but also important is the end-point of the physiologic action of vitamin D, namely, adequate plasma calcium and phosphate ions that provide the raw materials for bone mineralization.

3.2 Populations at risk for vitamin D deficiency

3.2.1 Infants

Infants constitute a population at risk for vitamin D deficiency because of relatively large vitamin D needs brought about by their high rate of skeletal growth. At birth, infants have acquired in utero the vitamin D stores that must carry them through the first months of life. A recent survey of French neonates revealed that 64% had 25-OH-D values below 30 nmol/l, the lower limit of the normal range (15). Breast-fed infants are particularly at risk because of the low concentrations of vitamin D in human milk (16). This problem is further compounded in some infants fed human milk by a restriction in exposure to ultraviolet (UV) light 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 6 months of their life indoors and therefore have little opportunity to synthesize vitamin D in their skin during this period. Consequently, although vitamin D deficiency is rare in developed countries, sporadic cases of rickets are still being reported in many northern cities but almost always in infants fed human milk (17–20).

Infant formulas are supplemented with vitamin D at levels ranging from 40 international units (IU) or 1 mg/418.4 kJ to 100 IU or 2.5 mg/418.4 kJ, that provide approximately between 6 mg and 15 mg of vitamin D, respectively. These amounts of dietary vitamin D are sufficient to prevent rickets.

3.2.2 Adolescents

Another period of rapid growth of the skeleton occurs at puberty and increases the need not for the vitamin D itself, but for the active form 1,25-(OH)₂D. This need results from the increased conversion of 25-OH-D to 1,25-(OH)₂D in adolescents (21). Unlike infants, however, adolescents usually spend more time outdoors and therefore usually are exposed to levels of UV light sufficient for synthesizing vitamin D for their needs. Excess production of vitamin D in the summer and early autumn months is stored mainly in the adipose tissue (22) and is available to sustain high growth rates in the winter months that follow. Insufficient vitamin D stores during this period of increased growth can lead to vitamin D insufficiency (23).

3.2.3 Elderly

Over the past 20 years, clinical research studies of the basic biochemical machinery for handling vitamin D have suggested an age-related decline in many key steps of vitamin D action (24), including the rate of skin synthesis, the rate of hydroxylation (leading to the activation to the hormonal form),

and the response of target tissues (e.g. bone) (25). Not surprisingly, a number of independent studies from around the world have shown that there appears to be vitamin D deficiency in a subset of the elderly population, characterized by low blood levels of 25-OH-D coupled with elevations in plasma PTH and alkaline phosphatase (26). There is evidence that this vitamin D deficiency contributes to declining bone mass and increases the incidence of hip fractures (27). Although some of these studies may exaggerate the extent of the problem by focusing on institutionalized individuals or inpatients with decreased sun exposures, in general they have forced health professionals to re-address the vitamin D intake of this segment of society and look at potential solutions to correct the problem. Table 3.1 presents the findings of several studies that found that modest increases in vitamin D intakes (between 10 and 20 µg/day) reduce the rate of bone loss and the incidence of hip fractures.

These findings have led several agencies and researchers to suggest an increase in recommended vitamin D intakes for the elderly from 2.5–5 µg/day to a value that is able to maintain normal 25-OH-D levels in the elderly, such as 10–15 µg/day. This vitamin D intake results in lower rates of bone loss and is proposed for the middle-aged (50–70 years) and old-aged (>70 years) populations (33). The increased requirements are justified mainly on the grounds of the reduction in skin synthesis of vitamin D, a linear reduction occurring in both men and women that begins with the thinning of the skin at age 20 years (24).

3.2.4 Pregnant and lactating women

Elucidation of the changes in calcitropic hormones occurring during pregnancy and lactation has revealed a role for vitamin D in the former but not definitively in the latter. Even in pregnancy, the changes in vitamin D metabolism which occur, namely an increase in the maternal plasma levels of 1,25-(OH)₂D (34) due to a putative placental synthesis of the hormone (35), do not seem to impinge greatly on the maternal vitamin D requirements. The concern that modest vitamin D supplementation might be deleterious to the fetus is not justified. Furthermore, because transfer of vitamin D from mother to fetus is important for establishing the neonate's growth rate, the goal of ensuring adequate vitamin D status with conventional prenatal vitamin D supplements probably should not be discouraged.

In lactating women there appears to be no direct role for vitamin D because increased calcium needs are regulated by the PTH-related peptide (36, 37), and recent studies have failed to show any change in vitamin D metabolites during lactation (38, 39). As stated above, the vitamin D content of human

TABLE 3.1
Randomized, controlled trials with dietary vitamin D supplements

Reference	Study group	n ^a	Age (years)		Regimen	Duration (years)	Results
			Mean	SD			
Dawson-Hughes et al., 1991 (28)	Healthy, postmenopausal women living independently	249	62	0.5	10 µg vitamin D + 400mg calcium	1.0	Reduced late wintertime bone loss from vertebrae Net spine BMD↑ No change in whole-body BMD
Chapuy et al., 1992 (29)	Healthy, elderly women living in nursing homes or in apartments for the elderly	3270	84	6	20 µg vitamin D + 1200mg calcium	1.5	Hip fractures 43% ↓ Non-vertebral fractures 32% ↓ In subset (n = 56), BMD of proximal femur 2.7% ↑ in vitamin D group and 4.6% ↓ in placebo group
Chapuy et al., 1994 (30) ^b						3.0	Hip fractures 29% ↓ Non-vertebral fractures 24% ↓
Dawson-Hughes et al., 1995 (31)	Healthy postmenopausal women living independently	261	64	5	2.5 µg or 17.5 µg vitamin D + 500mg calcium	2.0	Loss of BMD from femoral neck lower in 17.5 µg group (-1.06%) than in 2.5 µg group (-2.54%) No difference in BMD at spine
Lips et al., 1996 (32)	Healthy, elderly individuals living independently, in nursing homes, or in apartments for the elderly	2578 (1916 women, 662 men)	80	6	10 µg vitamin D		No difference in fracture incidence In subset (n = 248) of women from nursing homes, BMD 2.3% ↑ after 2 years

SD, standard deviation; BMD, bone mineral density; ↑, increase; ↓, decrease.

^a Number of subjects enrolled in the study.

^b Same study as Chapuy et al. (29) after a further 1.5 years of treatment.

Source: adapted, with permission, from reference (25).

milk is low (16). Consequently, there is no great drain on maternal vitamin D reserves either to regulate calcium homeostasis or to supply the need of human milk. Because human milk is a poor source of vitamin D, rare cases of nutritional rickets are still found, but these are almost always in breast-fed infants deprived of sunlight exposure (17–20). Furthermore, 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 (38). Thus, the current thinking, based on a clearer understanding of the role of vitamin D in lactation, is that there is little purpose in recommending additional vitamin D for lactating women. The goal for mothers who breastfeed their infants seems to be merely to ensure good nutrition and sunshine exposure in order to ensure normal vitamin D status during the perinatal period.

3.3 Evidence used for estimating recommended intakes

3.3.1 Lack of accuracy in estimating dietary intake and skin synthesis

The unique problem of estimating total intake of a substance that can be provided in the diet or made in the skin by exposure to sunlight makes it difficult to derive adequate total intakes of vitamin D for the general population. Moreover, accurate food composition data are not available for vitamin D, accentuating the difficulty in estimating dietary intakes. Whereas two recent United States national surveys have avoided even attempting this task, the second National Health and Nutrition Examination Survey (NHANES II) estimated vitamin D intakes to be 2.9 µg/day and 2.3 µg/day for younger and older women, respectively. A recent study of elderly women by Kinyamu et al. (40) concurred with this assessment, finding an intake of 3.53 µg/day.

Skin synthesis is equally difficult to estimate, being affected by such imponderables as age, season, latitude, time of day, skin exposure, and sunscreen use. In vitamin D-replete individuals, estimates of skin synthesis are put at around 10 µg/day (24, 41), with total intakes estimated at 15 µg/day (24).

3.3.2 Use of plasma 25-OH-D as a measure of vitamin D status

Numerous recent studies have used plasma 25-OH-D as a measure of vitamin D status, and there is a strong presumptive relationship of this variable with bone status. Thus, it is not surprising that several nutritional committees (e.g. the Food and Nutrition Board of the United States National Academy of Sciences' Institute of Medicine in conjunction with Health Canada) have chosen to use a biochemical basis for estimating required intakes and have used these estimates to derive recommended intakes (33). The method used involves the

estimation of the mean group dietary intake of vitamin D required to maintain the plasma 25-OH-D levels above 27 nmol/l, which is the level necessary to ensure normal bone health. Previously, many studies had established 27 nmol/l as the lower limit of the normal range (e.g. NHANES III [42]). This dietary intake of vitamin D for each population group was rounded to the nearest 50IU (1.25 µg) and then doubled to cover the needs of all individuals within that group irrespective of sunlight exposure. This amount was termed *adequate intake* (AI) and was used in place of the recommended dietary allowance (RDA), which had been used by United States agencies since 1941. The present Expert Consultation decided to use these figures as recommended nutrient intakes (RNIs) because it considered this to be an entirely logical approach to estimating the vitamin D needs for the global population.

Because many studies had recommended increases in vitamin D intakes for the elderly, it might have been expected that the proposed increases in suggested intakes from 5 µg/day (the RDA in the United States [43] and the RNI in Canada [44]) to between 10 and 15 µg/day (AI) would be welcomed. However, a recent editorial in a prominent medical journal attacked the recommendations as being too conservative (45). Furthermore, an article in the same journal (46) reported the level of hypovitaminosis D to be as high as 57% in a population of ageing (mean age, 62 years) medical inpatients in the Boston area.

Of course, such inpatients are by definition sick and should not be used to calculate intakes of healthy individuals. Indeed, the new NHANES III study (42) of 18323 healthy individuals from all regions of the United States suggests that approximately 5% had values of 25-OH-D below 27 nmol/l (see Table 3.2). Although the data are skewed by sampling biases that favour sample collection in the southern states in winter months and northern states in the summer months, even subsets of data collected in northern states in September give the incidence of low 25-OH-D in the elderly in the 6–18% range (47), compared with 57% in the institutionalized inpatient population (46) mentioned above. Ideally, such measurements in a healthy population should be made at the end of the winter months before UV irradiation has reached a strength sufficient to allow skin synthesis of vitamin D. Thus, the NHANES III study may still underestimate the incidence of hypovitaminosis D in a northern elderly population in winter. Nevertheless, in lieu of additional studies of selected human populations, it would seem that the recommendations of the Food and Nutrition Board are reasonable guidelines for vitamin D intakes, at least for the near future. This considered approach allows for a period of time to monitor the potential shortfalls of

TABLE 3.2

Frequency distribution of serum or plasma 25-OH-D: preliminary unweighted results from the third National Health and Nutrition Examination Survey, 1988–1994^a

Percentile	25-OH-D ^b (ng/ml) ^c
1st	7.6
5th	10.9
10th	13.2
50th	24.4
90th	40.1
95th	45.9
99th	59.0

^a Total number of samples used in data analysis: 18 323; mean: 25.89 ng/ml (± 11.08). Values are for all ages, ethnicity groups, and both sexes.

^b High values: four values between 90–98 ng/ml, one value of 160.3 ng/ml. Values <5 ng/ml (lowest standard) entered arbitrarily in the database as “3”.

^c Units: for 25-OH-D, 1 ng/ml = 2.5 nmol/l, 10 ng/ml = 25 nmol/l, 11 ng/ml = 28.5 nmol/l (low limit), 30 ng/ml = 75 nmol/l (normal), 60 ng/ml = 150 nmol/l (upper limit). Source: reference (42).

the new recommendations as well as to assess whether the suggested guidelines can be achieved, a point that was repeatedly raised about the vitamin D RDA.

3.4 Recommended intakes for vitamin D

In recommending intakes for vitamin D, it must be recognized that in most locations in the world in a broad band around the equator (between latitudes 42°N and 42°S), the most physiologically relevant and efficient way of acquiring vitamin D is to synthesize it endogenously in the skin from 7-dehydrocholesterol by sun (UV) light exposure. In most situations, approximately 30 minutes of skin exposure (without sunscreen) of the arms and face to sunlight can provide all the daily vitamin D needs of the body (24). However, skin synthesis of vitamin D is negatively influenced by factors which may reduce the ability of the skin to provide the total needs of the individual (24):

- latitude and season—both influence the amount of UV light reaching the skin;
- the ageing process—thinning of the skin reduces the efficiency of this synthetic process;

- skin pigmentation—the presence of darker pigments in the skin interferes with the synthetic process because UV light cannot reach the appropriate layer of the skin;
- clothing—virtually complete covering of the skin for medical, social, cultural, or religious reasons leaves insufficient skin exposed to sunlight;
- sunscreen use—widespread and liberal use of sunscreen, though reducing skin damage by the sun, deleteriously affects synthesis of vitamin D.

Because not all of these problems can be solved in all geographic locations, particularly during winter at latitudes higher than 42° where synthesis is virtually zero, it is recommended that individuals not synthesizing vitamin D should correct their vitamin D status by consuming the amounts of vitamin D appropriate for their age group (Table 3.3).

TABLE 3.3
Recommended nutrient intakes (RNIs) for vitamin D, by group

Group	RNI (μg/day) ^a
<i>Infants and children</i>	
0–6 months	5
7–12 months	5
1–3 years	5
4–6 years	5
7–9 years	5
<i>Adolescents</i>	
10–18 years	5
<i>Adults</i>	
19–50 years	5
51–65 years	10
65+ years	15
<i>Pregnant women</i>	5
<i>Lactating women</i>	5

^a Units: for vitamin D, 1 IU = 25 ng, 40 IU = 1 μg, 200 IU = 5 μg, 400 IU = 10 μg, 600 IU = 15 μg, 800 IU = 20 μg.

3.5 Toxicity

The adverse effects of high vitamin D intakes—hypercalciuria and hypercalcaemia—do not occur at the recommended intake levels discussed above. In fact, it is worth noting that the recommended intakes for all age groups are still well below the lowest observed adverse effect level of 50 μg/day and do not reach the “no observed adverse effect level” of 20 μg/day (33, 48). Outbreaks of idiopathic infantile hypercalcaemia in the United Kingdom in the post-World War II era led to the withdrawal of vitamin D fortification from all foods in that country because of concerns that they were due to hypervi-

taminosis D. There are some suggestions in the literature that these outbreaks of idiopathic infantile hypercalcaemia may have involved genetic and dietary components and were not due strictly to technical problems with over-fortification as was assumed (49, 50). In retrospect, the termination of the vitamin D fortification may have been counterproductive as it exposed segments of the United Kingdom community to vitamin D deficiency and may have discouraged other nations from starting vitamin D fortification programmes (50). This is all the more cause for concern because hypovitaminosis D is still a problem worldwide, particularly in developing countries, at high latitudes and in countries where skin exposure to sunlight is discouraged (51).

3.6 Recommendations for future research

Further research is needed to determine the following:

- whether vitamin D supplements during pregnancy have any positive effects later in life;
- whether vitamin D has a role in lactation;
- the long-term effects of high vitamin D intakes;
- whether dietary vitamin D supplements are as good as exposure to UV light;
- whether vitamin D is only needed for regulation of calcium and phosphate.

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