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VITAMIN D: A Growing Perspective

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□ *Vitamin D deficiency has been widely reported in all age groups in recent years. Rickets has never been eradicated in developed countries, and it most commonly affects children from recent immigrant groups. There is much evidence that current vitamin D guidelines for the neonatal period, 5–10 μg (200–400 IU)/day, prevent rickets at the typical calcium intakes in developed countries. The annual incidence of vitamin D-deficiency rickets in developed countries ranges between 2.9 and 7.5 cases per 100,000 children. The prevalence of vitamin D deficiency in mothers and their neonates is remarkable, and the results of one study suggest that third-trimester 25-hydroxyvitamin D (25(OH)D) is associated with fetal bone mineral accrual that may affect prepubertal bone mass accumulation. Beyond infancy, the evidence indicates that 5 μg (200 IU)/day of vitamin D has little effect on vitamin D status as measured by the serum 25(OH)D concentration. Two randomized clinical trials show that higher vitamin D intake improves one-year gain in bone density in adolescent girls. The functions of vitamin D extend beyond bone to include immune system regulation and anti-proliferative effects on cells. Early life vitamin D inadequacy is implicated in the risk of bone disease, autoimmune disease, and certain cancers later in life; however, long-term interventional studies do not exist to validate the widespread implementation of greater vitamin D consumption. Here we review the available data concerning vitamin D status and health effects of vitamin D in pregnancy through to and including adolescence.*

Keywords 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D, adolescence, childhood, growth, pregnancy, puberty, Vitamin D.

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Abbreviations **1α-OHase**, 1 α-hydroxylase or CYP27B1; **1,25(OH)2D**, 1,25-dihydroxyvitamin D; **24,25(OH)2D**, 24,25-dihydroxyvitamin D; **24-OHase**, 24-hydroxylase or CYP24A1; **25-OHase**, 25-hydroxylase or CYP27A1; **25(OH)D**, 25-hydroxyvitamin D; **AI**, adequate intake; **BALP**, bone-specific alkaline phosphatase; **BMC**, bone mineral content; **BMD**, bone mineral density; **CTx**, C-terminal telopeptide of type I procollagen; **DBP**, vitamin D binding protein; **DC**, dendritic cell; **DM**, diabetes mellitus; **DPD**, deoxypyridinoline; **EAE**, experimental allergic encephalomyelitis (mice); **FNB**, US Food and Nutrition Board; **GH**, growth hormone; **ICTP**, C-terminal cross-linked telopeptide of type I collagen; **IGF**, insulin-like growth factor; **IGFBP**, IGF binding protein; **LBW**, low birth weight; **MS**, multiple sclerosis; **NBW**, normal birth weight; **NOD**, non-obese diabetic (mice); **NTx**, N-telopeptide of type I collagen; **OC**, osteocalcin; **PBM**, Peak bone mass; **PBMCs**, peripheral blood mononuclear cells; **PICP**, procollagen I carboxy-propeptide; **PINP**, procollagen I amino-propeptide; **PTH**, parathyroid hormone; **PYD**, pyridinoline; **RA**, rheumatoid arthritis; **RANKL**, receptor activator of nuclear factor NFκB; **SGA**, small for gestational age; **SLE**, systemic lupus erythematosis; **UVB**, ultraviolet B; **VDR**, vitamin D receptor; **VDRE**, vitamin D response element; **Vitamin D2**, ergocalciferol; **Vitamin D3**, cholecalciferol.

I. INTRODUCTION

Vitamin D deficiency has long been recognized as a cause of bone disease, rickets in children and osteoporosis in adults. Since inadequate levels of vitamin D continue to be a problem worldwide for adults and children, recommended intake values for vitamin D need to be reevaluated by Health Canada and the US Food and Nutrition Board (FNB). Researchers tend to limit their analysis to a single group (mostly due to time and financial constraints); *e.g.*, recent studies have examined vitamin D status in pregnancy and lactation, as well as childhood and adolescence,[1–3] but none has spanned all four stages of development. Furthermore, although several studies have addressed the requirement of vitamin D in adults, including a safe upper limit,[4,5] no official consensus exists for optimal vitamin D status in children. Attempts to define an optimal 25(OH)D concentration have used various measurements: 25(OH)D concentrations demonstrated to suppress parathyroid hormone (PTH) levels to the lower end of normal; 25(OH)D levels that maximally increase calcium absorption; 25(OH)D levels associated with increased bone mineral density; or 25(OH)D concentrations associated with the reduction in the rate of bone loss, falling, and fractures. In adults, serum 25(OH)D concentrations >75 nmol/L are thought to be required for optimal bone health, with suggested intakes of vitamin D3 up to 25 μg (1,000 IU)/day (Table 1).[6] For infants and children, 25(OH)D concentrations <25 nmol/L remain the threshold of severe deficiency and the risk of rickets; yet this cut-off may be too conservative for other health aspects. Optimizing vitamin D nutritional status during periods of skeletal development has been proposed as a way of potentially enhancing the acquisition of bone mass in children for future prevention of osteoporosis. In addition, researchers also speculate that inadequate vitamin D status in youth may increase one's risk

TABLE 1 Estimates of the Minimum Serum 25(OH)D Level for Fracture Prevention in Adults and the Required Doses of Vitamin D3. (from Ref. 6, used with permission)

Investigator	Optimal 25 (OH)D (nmol/L)	Oral vitamin D3 dose needed to attain average optimal 25(OH)D	
		μg/day	IU/day
Lips	50	10–15	400–600
Holick	75	25	1,000
Heaney	80	40	1,600
Meunier	75	20	800
Vieth	70	25	1,000
Dawson-Hughes	80	25	1,000

of developing chronic disease later in life. Accordingly, the purpose of this paper is two-fold: first, it will review vitamin D metabolism and examine vitamin D status in various life stages; and second, it will examine the available supplementation data, the effects of vitamin D on bone accumulation, and the potential implications for both skeletal and extraskeletal health later in life.

II. VITAMIN D ENDOCRINE SYSTEM

A. Vitamin D Nutrition

Vitamin D nutritional status is unique among vitamins and minerals because it varies by environment; status is affected by latitude, culture, and food fortification legislation. The most important factor affecting vitamin D levels is sunshine exposure;[7] this effect is constantly demonstrated by seasonal variations in vitamin D levels. Seasonal variation is exhibited among many races, ages, and countries,[8–18] even at southerly latitudes such as Florida[19] and Italy.[20]

North American recommendations for vitamin D intake, as with most nutrients, vary according to age (Table 2). An adequate intake (AI) of 5 μg (200 IU/day; conversion: 1 μg = 40 IU) vitamin D is recommended for all persons under the age of 50 years and is considered likely to maintain adequate 25(OH)D in individuals with limited sun exposure. The evidence for this recommendation was primarily based on the vitamin D intake of healthy women regarded as having suitable serum 25(OH)D concentrations and not based on the response to a dose administered.[21] When the recommendations for vitamin D intake were undergoing deliberations, research priorities were generated, from which we now have more data, and consequently there is a need to revise these recommendations. An excellent question was raised by Hollis' group, who ask if it is logical that a small infant, a growing child, and

TABLE 2 Food and Nutrition Board Daily Dietary Reference Intakes (Adequate Intakes) for Calcium and Vitamin D Ref.[21]

Age (years)	Calcium mg/day	Vitamin D μg/day (IU/day)
0–0.5	210	5 (200)
0.5–1	270	5 (200)
1–3	500	5 (200)
4–8	800	5 (200)
9–18	1,300	5 (200)
19–50	1,000	5 (200)
51–70	1,200	10 (400)
>70	1,200	15 (600)
Pregnancy and Lactation		
≤ 18	1,300	5 (200)
19–50	1,000	5 (200)

an adult should all require the same amount of vitamin D given its role in skeletal development and maintenance.[22]

If one were to base the requirement for vitamin D on the serum concentrations of 25(OH)D found in a "healthy" population of individuals, we should look to individuals who obtain physiological values of 25(OH)D by natural means, *i.e.*, we should look to those individuals who are exposed, unblocked, to sunlight on a regular basis. Israeli lifeguards (n = 44), who worked in intense sunlight for at least 8 h/day, had mean 25(OH)D concentrations of 133 ± 84 nmol/L.[23] Puerto Rican farmers were found to have similar 25(OH)D concentrations at 133 ± 50 nmol/L.[24] Nigerian toddlers (2 months–5 years, n = 33) had mean serum 25(OH)D concentrations of 109 ± 74 nmol/L (unpublished data). These children and adults are excellent examples of normal physiological values of 25(OH)D as they are not restricted from sunlight. The 25(OH)D concentrations of lifeguards, farmers, and toddlers reflect those that can be maximally obtained from the environment. This suggests that normal 25(OH)D concentrations are >100 nmol/L.

Sufficient 25(OH)D concentration, attained solely from sunlight, is feasible in certain situations. Baseline mean 25(OH)D concentrations at the end of summer in a group of Lebanese schoolchildren (13.7 ± 2.1 years, n = 25) were found to be 100 nmol/L.[25] Children (4–8 years, n = 168) in the southern US had mean 25(OH)D concentrations of 93 nmol/L.[26] Similarly, holiday sunlight exposure in Tasmanian children was shown to produce 25(OH)D concentrations in the range of 80 nmol/L.[27,28] Artificial sunlight produces similar levels of vitamin D. A recent study examined 25(OH)D levels and bone mineral density in tanners, ≥1 time/week for ≥6 months, in comparison with people who never used a tanning bed. As expected, tanners had significantly higher mean 25(OH)D concentrations than non-tanners, 115.5 ± 8.0 compared to 60.3 ± 3.0 nmol/L, respectively ($p < 0.001$).[29] In addition, tanners had significantly higher bone mineral density (BMD) and z scores at the total hip than did non-tanners ($p = 0.04$).[30] In short, the

beneficial effects of an optimal vitamin D nutritional status can be obtained by either natural or artificial sunlight.

Given that total-body sun exposure during the summer is capable of producing 25(OH)D concentrations comparable to those obtained by oral intake of 250 μg (10,000 IU) of vitamin D[31] without any indication of adverse events or hypercalcemia, it appears that intakes of this magnitude can be tolerated. Toronto hospital workers taking 25 μg (1,000 IU)/day or 100 μg (4,000 IU)/day of cholecalciferol (vitamin D3) for five months had 25(OH)D concentrations that plateaued at 68.7 $\pm$ 16.9 (n = 15) and 96.4 $\pm$ 14.6 (n = 15) nmol/L, respectively.[32] This suggests that to maintain normal physiological values in winter at latitudes of 43°N, like Toronto, intakes of 100 μg (4,000 IU)/day are required.

Heaney *et al.* investigated the effects of vitamin D3 at 0, 25, 125, and 250 μg/day (or 0, 1000, 5000, and 10000 IU/day) over 20 weeks in 67 men.[33] Serum 25(OH)D concentrations in the placebo group decreased by 11.4 $\pm$ 17.7 nmol/L, whereas the vitamin D3 dose groups demonstrated an increase: 25 μg (1,000 IU)/day increased serum 25(OH)D by 12.0 $\pm$ 16.2, 125 μg (5,000 IU)/day by 91.9 $\pm$ 37.6 nmol/L and 250 μg (10,000 IU)/day by 159.4 $\pm$ 62.4 nmol/L.[33] Mathematical analysis of dose response demonstrated that for every one μg/day input of vitamin D3, 25(OH)D concentrations increased by 0.7 nmol/L.[33] In other words, to maintain baseline 25(OH)D concentrations of 70 nmol/L, 12.5 μg/day of vitamin D3 is required. This is more than double the current daily adequate intake recommended by Health Canada and the FNB. Further, to achieve and maintain a 25(OH)D level of 80 nmol/L (consensus requirement for optimal skeletal health) in adults requires a daily intake of 114 μg (4,560 IU), a dose more than double the tolerable upper level of intake for vitamin D, 50 μg (2,000 IU)/day. There was no incidence of hypercalcemia or adverse effects of supplementation noted in any of the studies listed above.

Similarly, none of the studies using vitamin D3 at doses as high as 50 μg (2,000 IU)/day show any evidence for vitamin D intoxication in children, defined as elevated serum calcium and high 25(OH)D concentrations.[25] In addition, 1,25-dihydroxyvitamin D (1,25(OH)2D) concentrations were unaffected in children supplemented with vitamin D.[25] Based on the evidence and given the high prevalence of hypovitaminosis D in children worldwide, a vitamin D dose of 50 μg (2,000 IU)/day is a reasonable replacement approach for children with insufficient vitamin D levels and who avoid sunshine.[34]

B. Vitamin D Metabolism

A vitamin, by definition, is required in small quantities in the diet, and a deficiency may result in disease. Vitamin D is required to prevent rickets in children and osteoporosis in adults. There are two forms of vitamin D: vitamin D2 (ergocalciferol) and vitamin D3. Vitamin D2 is of plant origin,

and vitamin D3 is the natural metabolite generated within the skin of humans and animals upon exposure to ultraviolet B (UVB) sunlight ($\lambda = 290–320$ nm). The two vitamin D molecules differ in structure; vitamin D2 has an extra double bond between carbons 22 and 23 and an additional 24-methyl group in comparison with vitamin D3. The two molecules also differ in their ability as supplements, vitamin D3 having been demonstrated to be two to three times more effective than vitamin D2.[35–37] When administered as a single dose of 50,000 IU of vitamin D2 or D3 and followed for 28 days [35] or as 4,000 IU/day given for two weeks,[36] the rise in serum 25(OH)D concentrations was more effective with vitamin D3 than D2. Recently, Holick *et al.* found no difference in the increase in 25(OH)D3 and 25(OH)D2 after 11 weeks of supplementation with either 1,000 IU/day of vitamin D3 or D2 or 500 IU/day of both.[38] This discrepancy from the more commonly obtained observation may reflect a relatively low dose and a lack of statistical power. The difference in binding affinity of vitamin D2 metabolites to vitamin D binding protein (DBP), being weaker than that for vitamin D3, would lead to a shorter plasma half-life and an increased rate of clearance from circulation.[37]

The vitamin D metabolic pathway (illustrated in Figure 1) consists of three major steps mediated by three different hydroxylase enzymes, all of which are cytochrome P450 enzymes that function as oxidases.[39] 7-dehydrocholesterol, the first metabolite in the vitamin D pathway and the

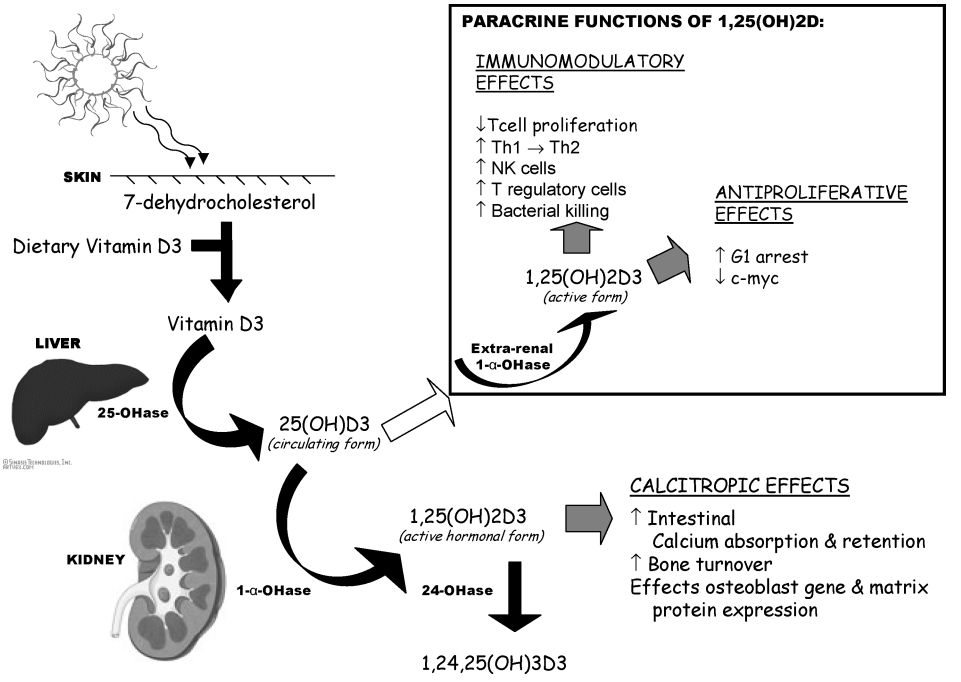

FIGURE 1 Vitamin D metabolism and actions.

immediate precursor in the biosynthesis of cholesterol, is located in the skin with the highest concentrations in the inner epidermis and can also be found in the oily secretions of sebaceous glands.[40] UVB rays stimulate the photoconversion of 7-dehydrocholesterol to vitamin D3. Vitamin D, dietary or synthesized, is taken up into the bloodstream where it binds DBP for transport. DBP is found in body fluids [41] and various tissues [42] and predominantly functions in the binding, solubilization, and serum transport of the vitamin D sterols,[43] though recent evidence suggests DBP may have roles in inflammation and the immune system.[44–46] It has been estimated that 10 mg/kg/day of DBP is produced in humans[47] with a half-life of 2.5 days,[48,49] and the total binding capacity of vitamin D metabolites is approximately 4,700 nmol/L.[50] DBP binds many of the vitamin D metabolites with different strengths; in relative affinities DBP has the highest affinity is for 25(OH)D and 24,25-dihydroxyvitamin D 24,25(OH)2D, less affinity for 1,25(OH)2D, and the lowest affinity for vitamin D.[41] In addition, DBP has stronger binding affinities for vitamin D3 metabolites than for vitamin D2.[51]

In the first hydroxylation reaction, vitamin D 25-hydroxylase (25-OHase), or CYP27A1, adds a hydroxyl group to produce 25(OH)D, the biologically inactive, major circulating metabolite of vitamin D. 25-hydroxylase was localized to the liver,[52] and it can also be found in the skin, kidney, and intestine. Four forms of 25-OHase have been identified, of which a hepatic microsomal enzyme appears to be the most physiologically active.[53] 25-OHase exhibits first-order kinetics, the rate of catalysis being proportional to the concentration of vitamin D available. Approximately 75% of circulating vitamin D is 25-hydroxylated in a single pass through the liver.[39] Circulating vitamin D is therefore readily converted to 1,25(OH)D, by the addition of a second hydroxyl group.

Once in the blood stream the DBP-25(OH)D complex is removed from the plasma by a variety of target tissues. This process releases most of the 25(OH)D into the tissue, whereas DBP is degraded. Deprivation studies in submarine excursions (sudden elimination of sunlight) have found the half-life of 25(OH)D to be approximately four weeks.[54,55] Vitamin D metabolites bound to DBP have limited access to target cells.[48] As confirmed by studies in mice, bound metabolites are less susceptible to hepatic metabolism and subsequent biliary excretion, which prolongs their half-life in the circulation.[56] The conversion of 25(OH)D to the active hormonal form of vitamin D, 1,25(OH)2D, the addition of a second hydroxyl group is catalyzed by 1-α-hydroxylase (1-αOHase; CYP27B1), primarily found in the kidneys.

Circulating concentrations of 1,25(OH)2D are believed to be controlled primarily at the level of 1-αOHase expression in the kidney (Figure 2). 1-αOHase expression is activated by PTH, which is secreted in response to low extracellular calcium concentrations.[57] Regulation of 1-αOHase expression is independently augmented by low calcium and phosphate signals.[58] Once activated, negative feedback inhibition by 1,25(OH)2D results in

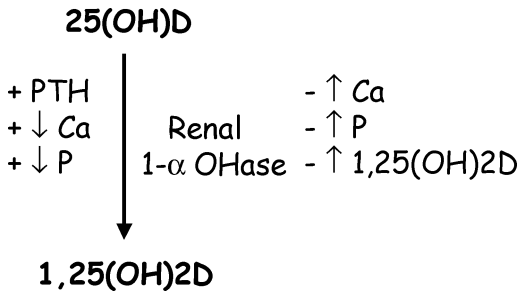

FIGURE 2 Hormonal regulation of renal 1-α-OHase. Regulation of 1-αOHase is either stimulated (+) or downregulated (−) under the influence of the hormones (PTH, parathyroid hormone; 1,25(OH)2D, 1,25-dihydroxyvitamin D) and minerals indicated (Ca, calcium; P, phosphorus).

down-regulation of 1-αOHase expression and prevents excess circulating 1,25(OH)2D concentrations. Hyperphosphatemia and hypercalcemia also negatively regulate 1α-OHase expression.

Circulating levels of 1,25(OH)2D are tightly regulated and involve reciprocal changes in the rates of synthesis and degradation.[59] Vitamin D metabolites are degraded by oxidation of the side chain which is catalyzed by vitamin D-24-hydroxylase (24-OHase; CYP24A1), producing calcitroic acid, a biologically inert metabolite.[60] 24-OHase is ubiquitously expressed in vitamin D target tissues and is highly inducible by 1,25(OH)2D. The effects of PTH are exerted in the kidney on the renal proximal tubular cells,[61] where 1-αOHase and 24-OHase are found. In response to hypocalcemia, PTH strongly induces 1-αOHase activity, stimulating 1,25(OH)2D production while simultaneously inhibiting 24-OHase activity.[62] In contrast, hypercalcemia depresses 1-αOHase activity and increases 24-OHase activity. Renal concentrations of both enzymes are significant determinants of serum 1,25(OH)2D.[63] Circulating concentrations of 1,25(OH)2D are therefore tightly regulated to maintain calcium homeostasis.

Due to the tight regulation of 1,25(OH)2D by serum calcium, phosphorus, and PTH, 1,25(OH)2D is not a good indicator of vitamin D status.[64] Serum 25(OH)D concentrations are the best indicator for determining adequacy because it represents the combined amounts of vitamin D synthesized in the skin and dietary sources.[65] Serum 25(OH)D levels are the accepted measure of vitamin D nutritional status.[21]

C. Absorption of Dietary Vitamin D3 and Distribution of Vitamin D3 in the Body

Dietary vitamin D absorption is dependent upon the presence of bile salts and occurs mainly in the jejunal segment of the small intestine, as seen in animal experiments using radioactively labeled vitamin D3 and vitamin D2.[66] Absorption occurs independent of vitamin D status.[66] Absorption of 25(OH)D is more rapid than that of vitamin D3,[67,68] however vitamins D3

and D2 produce an initial response that is similar.[69] Vitamin D3 prepared with medium chain triglyceride capsules requires the presence of fat from a standard meal to stimulate bile and produce absorption rates comparable with vitamin D3 suspended in peanut oil.[70]

From the small intestine, vitamin D3 and 25(OH)D are transferred into the lymph where vitamin D3 is found mainly in chylomicrons and 25(OH)D in the plasma protein fraction.[66,67] Vitamin D3 and 25(OH)D concentrations rise in the plasma, peaking approximately 3 h after UVB exposure, followed by vitamin D3 accumulation in tissues throughout the body.[71–73] In humans and rats, the primary storage sites of unmetabolized vitamin D3 are adipose tissue and skeletal muscle.[72,73] In rat experiments, UVB irradiation resulted in the rapid conversion of vitamin D3 to 25(OH)D and, once plasma 25(OH)D concentrations had reached a maximum, vitamin D3 concentrations in adipose tissue and skeletal muscle also began to rise rapidly.[71] Using radio-labeled vitamin D3, the most constant amounts of vitamin D3 were found in adipose tissue of rats at 10–12% of the administered dose, whereas skeletal muscle contained approximately 2% of the administered dose.[74] Human data are similar, with the highest concentration of vitamin D3 found in fatty tissues. However, due to the greater total body mass of skeletal muscle, the total amount of vitamin D3 stored in skeletal muscle is nearly equivalent to that in adipose tissue.[73] The half-life of vitamin D3 in adipose tissue of rats and humans has been found to be around 80 days.[73–75]

The total amount of recoverable vitamin D3 from serum and tissue stores in these studies accounts for only 25–50% of the administered dose.[71,73] In humans, the maximum rate of excretion (measured by the amount of radioactivity in the bile) occurred within 36–40 h after administration of vitamin D3.[73] It has been estimated that 75% of a single dose of vitamin D3 is excreted without ever being stored in tissue or converted to 25(OH)D.[43]

D. Mechanism of Action

The biological effects of 1,25(OH)2D are primarily mediated through interaction with the cytosolic vitamin D receptor (VDR). VDR is ubiquitously expressed and found in the classic vitamin D target organs (intestine, bone, kidney, and parathyroid glands)[76] as well as in numerous cells and organs not involved in calcium regulation (Table 3). Free 1,25(OH)2D enters most cells by diffusion.[50] In some cell types, such as the liver or kidney, that possess megalin, 1,25(OH)2D can be taken up by facilitated diffusion.[33] 1,25(OH)2D enters the cell where it is bound by VDR. The complex formed binds the retinoic acid receptor forming a heterodimer [77,78] and acts on vitamin D response elements (VDREs) found in target genes to initiate gene transcription, exerting its effects by either up-regulating or down-regulating gene products.[79]

TABLE 3 Tissue VDR Expression 1-α-OHase Activity

Tissue	VDR expression	1-α-OHase activity
Intestine	+[76]	
Bone	+[76]	
Kidney	+[76,459]	+[460]
Parathyroid	+[461]	+[462]
Thyroid	+[463]	
Adrenal Medulla	+[464]	
Adrenal Cortex		ND[460]
Pituitary	+[465]	
Keratinocytes	+[466,467]	+[460]
Colon epithelials	+[468]	+[460]
Pancreatic islets	+[469]	+[460]
Decidua	+[460]	+[460]
Placenta	+[460]	+[460]
Neurons	+[470,471]	+[470]
Glia	+[470]	+[470]
Endothelial cells	+[472]	+[472,473]
Breast tissue	+[474,475]	+[475]
Prostate	+[476]	+[477]
Skeletal muscle	+[478,479]	
Heart	+[480]	ND[481]
Dendritic cells	+[482]	+[483]
Macrophages	+[484]	+[485–487]
Monocytes	+[488]	+[489]
T lymphocytes	+[489,490]	+[491]
B lymphocytes	+[489]	
Liver	+[492]	ND[493]

ND indicates *not detected.*

The "classic" functions of 1,25(OH)2D are involved in calcium homeostasis and bone metabolism. It is well known that 1,25(OH)2D increases intestinal absorption of both calcium and phosphorus. PTH, released in response to low calcium concentrations, stimulates the activity of renal 1-αOHase. As a result, synthesis of 1,25(OH)2D is increased, thereby promoting increased calcium absorption. In addition, 1,25(OH)2D stimulates renal retention of calcium to promote bone tissue mineralization. At a molecular level, 1,25(OH)2D alters the expression of numerous osteoblast genes and the synthesis of matrix proteins that regulate bone mineralization and are essential for bone growth.[80] 1,25(OH)2D also directly stimulates osteoblast activation of osteoclasts through the induction of the receptor activator of nuclear factor NFκB (RANKL), thus activating bone resorption,[81] which is fundamental to bone remodeling and skeletal growth. Chronically elevated 1,25(OH)2D concentrations result in prolonged bone resorption and have only detrimental effects on bone. Overall, circulating 1,25(OH)2D acts to maintain calcium homeostasis, even in mineral-deficient states.

Vitamin D functions extend beyond those of bone mineralization and calcium homeostasis. In fact, 1,25(OH)2D regulates over 60 genes.[53] 1,25(OH)2D is involved in cellular growth and differentiation.[82] Many genes

in prostate, colon, and breast cancer cells are positively (*i.e.*, stimulation of differentiation) or negatively (*i.e.*, suppression of proliferation) regulated through the VDR.[83] In the immune system 1,25(OH)2D regulates the type of response produced by an antigenic stimulant. 1,25(OH)2D can down-regulate a pro-inflammatory Th 1 lymphocyte response by suppressing the antigen-presenting capacity of macrophages and dendritic cells (DC), to promote an anti-inflammatory Th 2 lymphocyte response.[84] The presence of both 1-αOHase and VDR in extra-renal tissues (Table 3) allows for the conversion of 25(OH)D to 1,25(OH)2D and local use of 1,25(OH)2D. It is speculated that improving vitamin D nutritional status (increasing 25(OH)D substrate concentrations) may allow for better regulation of systems such as the immune system, which may be beneficial in certain disease states. Widespread co-expression of VDR and 1-αOHase emphasizes a putative role for 1,25(OH)2D as a local agent with diverse physiological functions, and, by extension, vitamin D nutritional status is likely to have many health impacts.

III. PREGNANCY, LACTATION, AND INFANCY

A. Pregnancy

Skeletal growth is a complex process that begins *in utero* and continues into early adulthood. Calcium and vitamin D play major roles in skeletal development and are especially important during periods of rapid growth. As a requirement of skeletal growth, fetal calcium accretion starts mid-gestation and increases into the third trimester when the greatest amount of fetal calcium accretion occurs.[85] For instance, fetal accrual of calcium has been found to increase from approximately 50 mg/day at 20 weeks gestation to 330 mg/day at 35 weeks gestation, averaging around 250 mg/day for the third trimester.[86,87] It is important that the maternal supply of minerals across the placenta is sufficient to match these accretion rates.

To accommodate increased requirements for calcium, maternal intestinal absorption of calcium rises and reaches a maximum in the last trimester. Throughout this process, elevated serum 1,25(OH)2D concentrations, with appropriate dietary calcium intakes, increase calcium absorption to account for calcium transfer to the fetus.[88] It has been noted that calcium absorption efficiency approximately doubles in pregnancy, starting as early as 12 weeks of gestation.[89,90]

In addition to increased calcium absorption, the maternal skeleton may act as a reservoir of calcium. Indeed, both formation[91] and resorption [91,92] indices increase from early gestation by 50–200% by the end of pregnancy,[91–95] indicating a dynamic response by the maternal skeleton to fetal growth demands. Although changes in serum and urine bone turnover markers do occur in pregnancy, it is unclear whether these result in a net deficit in maternal skeletal mineral.[90]

Maternal adaptation to increased calcium requirements is modulated by increased concentrations of 1,25(OH)2D. 1,25(OH)2D levels are increased from the beginning of pregnancy[90,92,93,96–99] through up-regulation of renal and placental 1-α-OHase and extrarenal synthesis.[92] In fact, 1,25(OH)2D concentrations during the second trimester can more than double in comparison with pre-pregnancy values and can increase by 100% during the third trimester.[92] Levels of DBP also increase. During the third trimester, there is an increase in the amount of free 1,25(OH)2D in both mother and fetus,[100] suggesting that increased concentrations of free 1,25(OH)2D are important to fetal growth and development. Increased serum 1,25(OH)2D concentrations have been positively associated with intestinal calcium absorption during late pregnancy,[92,93] yet the mechanisms by which 1,25(OH)2D concentrations are elevated have yet to be elucidated. Typically, increased PTH would account for increased 1,25(OH)2D levels, yet PTH levels are not elevated during pregnancy[92,93,101] and therefore cannot be the stimulus. Zehnder *et al.* have demonstrated that expression of 1-αOHase was ~1000-fold higher in decidua and 80-fold higher in placental tissue during the first and second trimesters when compared with third trimester values.[102] Further, a proportion of circulating 1,25(OH)2D has been shown to be derived from maternal decidual cells.[103–105] 1,25(OH)2D receptors are up-regulated as well. VDR expression has the highest levels in first-trimester decidua.[102,106] The mechanisms by which maternal 1,25(OH)2D levels increase are not yet entirely understood nor are all the functions of 1,25(OH)2D during pregnancy.

It is known that vitamin D transport occurs mainly through the placenta in the form of 25(OH)D.[96,107,108] At birth, serum 25(OH)D concentrations in the mother correlate with those of the fetus,[109–116] with cord levels approximating 80% of maternal concentrations.[113] Adequate maternal vitamin D status during pregnancy is important for neonatal calcium metabolism. In fact, neonatal calcium metabolism can be negatively affected by a maternal deficiency of vitamin D. In premature infants, bone demineralization can occur and can be corrected with vitamin D supplementation.[117] Further, maternal vitamin D deficiency can result in maternal secondary hyperparathyroidism, which may lead to transitory hypocalcemia and hyperparathyroidism in the neonate.[118,119]

Several studies have reported that infants born to mothers with insufficient vitamin D stores during pregnancy have low serum calcium concentrations in cord blood or during the first week of life.[119–123] Paunier *et al.* examined vitamin D and calcium status during the winter months of 40 Swiss mothers and their term infants at delivery.[122] Calcium concentrations in infants on day four were found to be significantly lower among infants whose mothers consumed <3.75 μg (150 IU)/day of vitamin D, compared with those whose mothers consumed >12.5 μg (500 IU)/day of vitamin D during pregnancy. Namgung *et al.* demonstrated that bone mineral content (BMC) of winter-born infants in Korea was higher than that of summer-born

infants and speculate that maternal vitamin D status during early pregnancy was the influencing factor on fetal bone mineralization.[124] MacLennan *et al.* found that 25(OH)D concentrations fell progressively with each trimester of pregnancy,[125] which supports increased conversion to 1,25(OH)2D during the last trimester. Fetal calcium concentrations and bone growth are likely influenced by maternal vitamin D status.

B. Vitamin D Status During Pregnancy

Reports of maternal and newborn vitamin D insufficiency are abundant and demonstrate a need for increased vigilance concerning vitamin D nutrition. Among the reports of vitamin D status, many different thresholds are used to distinguish deficiency, insufficiency, and sufficiency. Typically, serum concentrations <25 nmol/L are associated with rickets, signaling severe deficiency, yet optimal levels have not been established. Henriksen *et al.* found that of pregnant Pakistani women surveyed (n = 38) in Oslo, 83% had 25(OH)D concentrations <30 nmol/L.[126] Grover and Morley found that 80% of veiled or dark-skinned pregnant women (n = 82) had 25(OH)D concentrations <22.5 nmol/L at their first antenatal clinic visit in Melbourne, Australia.[127] In New Zealand, 87% of women screened at their first antenatal visit for vitamin D deficiency had serum 25(OH)D concentrations <50 nmol/L and 61.2% were <25 nmol/L.[128] In the Netherlands, mean serum 25(OH)D was 15 ± 12 nmol/L in Turkish women at 12 weeks of pregnancy, 20 ± 13 nmol/L in Moroccan women, and 53 ± 22 nmol/L in Western women; levels were below the detection limit in 22% of Turkish women.[129] Similarly, 55% of non-European women (mostly Turkish and Moroccan) living in the Netherlands were found to be severely vitamin D deficient (<20 nmol/L), and 54% of infants born to these mothers had 25(OH)D concentrations <13 nmol/L.[130] In Southern France, 34% of pregnant women (n = 59) were found to have 25(OH)D concentrations <25 nmol/L, and 32% of these women were hypocalcemic.[131] Sachan *et al.* reported an incidence of hypovitaminosis D of 84% in pregnant Indian women.[132] In Turkey, Pehlivan *et al.* found that of 78 women in the third trimester of pregnancy, 25(OH)D concentrations were <40 nmol/L in 94.8 % and <25 nmol/L in 79.5% of the women surveyed.[133] Twenty-five percent of infants born to these mothers (n = 65) had 25(OH)D concentrations of <40 nmol/L.[133] Non-European ethnic minorities (n = 160) in a South Wales clinic were surveyed at their first antenatal visit, and 50% were found to have vitamin D concentrations <20 nmol/L.[134] In Pittsburgh, USA, 29.2% of black women (n = 200) and 45.6% of their neonates had 25(OH)D concentrations <37.5 nmol/L.[135]

Many others have reported vitamin D deficiency over the past 25 years.[114,125,136–150] Recently, Schroth *et al.* reviewed the prevalence of vitamin D deficiency during pregnancy.[151] Out of 76 studies included, 35 reported deficient mean, or median, concentrations of 25(OH)D.[151] In addition,

vitamin D levels vary throughout the course of gestation. Ainy *et al.* found that of the women observed, 60% during the first trimester, 48% during the second trimester, and 47% during the third trimester had 25(OH)D concentrations <50 nmol/L.[152] Others have also shown increases in vitamin D status during pregnancy.[125,153,154] One study found that 25(OH)D concentrations significantly increased by trimester in patients who delivered in autumn, whereas those who delivered in spring had 25(OH)D concentrations that had significantly decreased.[110] In summary, consistently high proportions of pregnant women display vitamin D concentrations considered insufficient by all standards (<50 nmol/L). Despite recommendations from Health Canada and the Canadian Pediatric Society (www.cps.ca), vitamin D deficiency is an issue in Canada, especially for those of darker skin living in northern regions. The negative effects of maternal vitamin D insufficiency include deleterious effects on maternal and/or the fetal skeleton and may predispose the child to a number of chronic diseases.[155] Studies investigating vitamin D requirements in pregnancy are needed to derive guidelines for general practitioners, midwives, and obstetricians involved in antenatal care and for public education.

C. Maternal Vitamin D Status During Pregnancy and Impact on Neonatal Anthropometric and Skeletal Parameters

Rickets or osteopenia may present in the newborn infant in cases of decreased skeletal mineralization due to severe vitamin D deficiency. The results of several observational studies suggest that an infant's bone mass may be related to the vitamin D status of the mother.[156]

Because the fetus utilizes maternal sources of 25(OH)D to synthesize 1,25(OH)2D, maternal vitamin D requirements increase during pregnancy. Maternal 25(OH)D concentrations tend to fall during the third trimester, especially if this occurs during winter.[125] Namgung *et al.* found that Korean infants born in winter months have lower total body BMC at birth, lower cord 25(OH)D concentrations, and higher rates of resorption markers.[157] Infant total body BMC in this cohort was positively correlated with cord serum 25(OH)D and inversely correlated with the C-terminal cross-linked telopeptide of type I collagen (ICTP), a biomarker of bone formation. Thus, low infantile vitamin D status is associated with low BMC values. North American studies have demonstrated lower BMC in infants born in summer (after a winter pregnancy with no vitamin D synthesis) compared with winter-born neonates (after a summer pregnancy with higher vitamin D status).[124,157,158] The difference in BMC was postulated by the authors to be influenced by the markedly different maternal vitamin D3 status in summer months compared with winter when there is little to no production of vitamin D. Specker *et al.* demonstrated a possible relationship between vitamin D deficiency and impaired fetal bone ossification in which neonatal wrist ossification centers were less likely to be found among infants born in the

spring compared with those born in the fall.[159] The authors speculated that the low rate of ossification centers in the spring could be attributed to low maternal vitamin D status during the winter months. Some have suggested that the amounts of maternal vitamin D lost to the fetus or newborn, either across the placenta or into breast milk, are inconsequential and unlikely to compromise the vitamin D status of the mother.[160] This debate may be moot, since higher maternal 25(OH)D concentrations appear to protect against pre-eclampsia,[161] and this strengthens the case that vitamin D status must not be neglected during pregnancy.

Contributions of early growth to BMC[162] suggest that the trajectory of bone growth may be modified *in utero*. Indeed, a growing body of evidence supports the importance of maternal nutrition and fetal hormonal milieu for neonatal and adult health in general, and bone health in particular, a phenomenon known as fetal programming.[163] Morley *et al.* showed that maternal hypovitaminosis D in the third trimester of pregnancy was associated with reduced offspring knee-heel length at birth.[164] Similarly, in a longitudinal study of 198 children, Javaid *et al.* demonstrated that maternal vitamin D insufficiency or deficiency in late pregnancy was associated with reduced whole body and spine BMC of their nine-year-old children.[165]

Vitamin D consumption during gestation may indirectly affect infant birth weight. Mannion *et al.* compared birth weights of infants born to women with low milk consumption (<250 mL/day), regarded as those least likely to consume a daily vitamin D intake of 5 μg (200 IU), with those born to women who did not restrict their consumption of milk.[166] Women who did not restrict their milk consumption were found to have an average consumption of 1864 $\pm$ 497 mg/day of calcium and 13.1 $\pm$ 4.5 μg (524 $\pm$ 180 IU)/day of vitamin D.[166] The birth weight of the infants born to women who consumed <250 mL/day was lower when compared with those born to women who consumed more milk. Although 25(OH)D concentrations were not measured in this study, it is known that Canadian mothers and their infants have high rates of vitamin D deficiency due to seasonal limitations on vitamin D production. Intake of milk, and therefore vitamin D and calcium, during pregnancy was associated with increased infant birth weight. Epidemiological studies have demonstrated an association between infant weight at one year and BMC in adulthood,[167] suggesting that vitamin D status is linked to infant birth weight.

D. Vitamin D Supplementation During Pregnancy

The current AI for vitamin D during pregnancy in Canada and the United States remains at 5 μg (200 IU)/day,[21] despite the findings of insufficiency during pregnancy by several studies (Table 4). Recently, a Cochrane review examining vitamin D supplementation during pregnancy [168] identified seven studies,[109,111,112,169–172] of which four reported clinical

TABLE 4 Summary of Vitamin D Supplementation Studies During Pregnancy

Reference	Location & Latitude	Population	Vitamin D Protocol	25(OH)D (nmol/L) Initial	25(OH)D (nmol/L) Endpoint	Other outcomes
Marya[172]	Rohtak India 28.5°	Hindu women (n = 120)	Recruited for third trimester D+: 1,200 IU/day (n = 25) or 600,000 IU once each in seventh & eightth months (n = 20) D-: placebo (n = 75) *Randomized trial*	NM	NM	Maternal & cord Ca higher & alk phos lower in D+ (600,000 IU) versus D− group.
						Birth weight greater in both D+ compared with D- group.
Marya[494]	Rohtak, India 28.5°	Asian-Indian immi-grantsrecruited (n = 200)	Recruited for third trimester D+: 600,000 IU once each during seventh & eightth months (n = 200) D-: placebo (n = 100) *Randomized trial*	NM	NM	Maternal & cord Ca & P higher & alk phos lower in D+ vs D- size greater.
						Infant birth weight and in D+ versus D-group.
Delvin[109]	Lyon, France 45°N	French women (n = 120)	Recruited for third trimester D+: 1,000 IU/day (n∼15) D−: placebo (n∼15) *Randomized trial*	∼ 25	D+: 65	Cord 1,25(OH) 2D lower in D+ versus D-group @ birth.
					D-: 32.5	
				Infants at birth:		
					D+: 45	
					D-: 17.5	
Brooke[111]	London, England 51°N	Asian immigrants (n = 129) from: India, Bangladesh, Pakistan, Sri Lanka, Mauritius, East Africa	Recruited @ 12 wks gestation D+: 1,000 IU/day D2 (n = 59) D−: placebo (n = 67) *Randomized, double-blinded rial*	20.1	D+: 16.8	Five cases of symptomatic hypocalcemia, all in D-group.
					D−: 16.2	Small for Gestational Age infants: 19 in D− versus 9 in D+ group.
				Infants at birth:		
				D+: 137.9		
				D−: 10.2		

Brown[111]	London, England 51°N	Indian or Pakistani (n = 113)	Recruited @ 28 wks gestation D+: 1,000 IU/day D2 (n = 39) D−: placebo (n = 53) *Randomized trial*	20.1	D+: 176.1 D−: 16.0	Cord calcium levels higher in D+ group by 3 days and significantly higher by 6 days after birth.
Mallet[173]	Northwest of France ~45-48°N	French women (n = 160)	Recruited for 3rd trimester D+: 1,000 IU/day D2 (n = 21) or 200,000 IU once in 7^{th} mo (n = 27) D-: placebo (n = 29) *Randomized trial*	9.5	D+: 25	No difference in Ca or 1,25(OH)2D between D+ & D−. Neonatal Ca & birth weights similar for D+ & D- groups. 12.5–15 nmol/L increase in maternal & cord 25(OH)D.
Datta[134]	Cariff, Wales, UK 52°	English women (n = 160)	Antenatal 25(OH)D screening, deficient women (n = 80) given 800–1,600 IU/day vitamin D *Intervention trial*	14.5	D+: 28.0	PTH levels unchanged after supplementation with vitamin D.
Cockburn[112]	Edinburgh, Soctland 55°N	Scottish women (n = 1,139)	recruited @ 12wk gestation D+: 400 IU/day (n = 506) D−: placebo (n = 633) *Quasi-randomized trial*	D+: 39.0 D−: 32.5 **Infants at birth**: D+: 28.0 D−: 20.0		Five cases of symptomatic hypocalcemia, all in D− group. 400 IU/day did not significantly increase 25(OH)D in mothers or infants @ term.

outcomes.[111,112,170,171] Due to the limited number of studies conducted, their small samples sizes, compliance issues, and the poor quality of the data due to lack of randomized, placebo-controlled trials, there was insufficient evidence to evaluate the effects of vitamin D supplementation during pregnancy. However, the available data do support the need for an upward revision of vitamin D intake guidelines for pregnant women.

1. Impact of Vitamin D Supplementation During Pregnancy on Maternal 25(OH)D Levels

The AI during pregnancy is insufficient to increase maternal 25(OH)D concentrations or maintain them in an optimal range. Cockburn *et al.* supplemented mothers during the third trimester of pregnancy with 10 μg (400 IU)/day of vitamin D2 (n = 506) or no supplementation (n = 633).[112] Neonatal hypocalcemia was more frequent in infants born in the unsupplemented group,[112] but there was no significant increase in maternal or infant 25(OH)D concentrations at term, suggesting that this level of supplementation may be helpful albeit not sufficient. Vitamin D supplementation at intakes more than double current recommendations is not sufficient to raise low 25(OH)D concentrations in expectant mothers. Mallet *et al.* supplemented women with 25 μg (1,000 IU)/day of vitamin D2 during the third trimester of pregnancy with no significant increase in maternal or cord 25(OH)D concentrations.[173] Datta *et al.* supplemented vitamin D-deficient mothers with 20–40 μg (800–1600 IU)/day of vitamin D for the duration of their pregnancy.[134] After vitamin D supplementation, mean 25(OH)D concentrations did increase from 15 nmol/L at the beginning of pregnancy to 27 nmol/L at term, but higher concentrations are required before repletion can be accomplished. The mothers that started off with inadequate vitamin D status were still deficient at the end of their pregnancy, even after supplementation with 20–40 μg (800–1600 IU)/day of vitamin D. The above studies suggest that >25 μg (1,000 IU)/day of vitamin D2 is required to replete the vitamin D status of deficient mothers. A confounding factor in these studies lies in the choice of vitamin D supplement used, vitamin D3 being more effective at raising serum 25(OH)D concentrations than vitamin D2,[37] and the chosen dose may have been too low. Moreover, compliance may have been an issue in the above studies. As detailed below, such doses may also optimize infant anthropometric measurements, and, because of the tight relationship between body weight, height, and bone mass, they may also ultimately affect infant skeletal parameters.

2. Impact of Vitamin D Supplementation During Pregnancy on Neonatal Outcomes

For an efficient increase in calcium absorption during pregnancy, a corresponding increased requirement for vitamin D is present. It has

been demonstrated that 1,25(OH)2D levels increase by 50% in the third trimester of pregnancy,[91,92,174] which is consistent with peak fetal calcium accretion.[92,93] Maternal vitamin D insufficiency may lead to inadequate calcium absorption. Animal experiments have demonstrated that low prenatal vitamin D3 status negatively influences fetal skeletal growth.[175] Brooke *et al.* found that infants born to mothers who had not received vitamin D supplementation had larger fontanelles when compared with those born to mothers supplemented with 25 μg (1,000 IU)/day of vitamin D2.[111] This finding is consistent with impaired ossification of the skull. Administration of 25 μg (1,000 IU)/day of vitamin D to vitamin D-deficient Asian women during the third trimester resulted in a lower proportion of neonates with low birth weight compared to the group who received placebo.[170] In the same study, infants of vitamin D-supplemented mothers were also significantly longer and heavier at 12 months than those of control mothers.[171] Similarly, Marya *et al.* showed that infants of mothers who received 15,000 μg (600,000 IU) of vitamin D during the seventh and eighth months, of pregnancy had the highest birth weight.[172] In contrast, Congdon *et al.* found no association between infant BMC values and vitamin D supplementation during pregnancy.[123] Finally, in the study of Mallet *et al.*, low birth weight was noted in infants of mothers supplemented with vitamin D.[173] Findings from the above studies are inconclusive since the optimal dose and period of supplementation are unknown (Table 4).

In summary, maternal hypovitaminosis D during pregnancy is quite common in apparently healthy young women. This finding is particularly worrisome because the fetus relies on maternal sources of 25(OH)D to synthesize calcitriol, and thus low maternal 25(OH)D may lead to reduced osteoblastic activity and long bone growth of the fetus. In a sample taken in Manitoba, Canada, Lebrun *et al.* found that 76% of mothers and 43% of infants had 25(OH)D concentrations <25 nmol/L.[176] Such deficiencies have been linked in some studies to delayed intrauterine skeletal growth after birth and may translate into an increased risk of osteoporotic fracture later in life. Furthermore, in view of the large number of target genes of which vitamin D induces the transcription of[177] vitamin D is likely to be an important factor in the regulation of the physical, and possibly mental development of the fetus (see section G below).

E. Breastfeeding: Vitamin D Content in Human Milk and Supplementation During Lactation

Calcitropic hormones play a role during lactation. In the later stages of lactation, increases in both PTH and 1,25(OH)2D have been observed, including after cessation of breastfeeding,[93,178,179] though this finding has been inconsistent.[180] Even greater elevations in PTH and 1,25(OH)2D have been seen in mothers nursing twins compared with mothers nursing

a single infant.[181] Bone turnover is also elevated in the first months of lactation,[178,179,182] and significant reductions in maternal BMC have been associated with lactation, an effect that is reversed after breastfeeding stops.[89] The skeletal effects of lactation are transient for the mother, but may not be so easily overcome for her infant. Maternal 25(OH)D level is the only source of fetal and neonatal 25(OH)D, and thus the only source of 1,25(OH)2D. Low maternal 25(OH)D levels are associated with lower anthropometric measures of the neonate and infant[164] and lower bone-mass parameters of offspring several years later.[165]

Mothers with low 25(OH)D concentrations are ill equipped to provide sufficient 25(OH)D stores for their babies. Low maternal vitamin D status is shared with the infant through the placental circulation prenatally and then through breastfeeding. Breastfed infants of mothers with low 25(OH)D, who are unsupplemented and with little sunlight exposure, are at high risk of developing vitamin D deficiency or rickets.[143,183] The vitamin D content of human breast milk has been found to be quite low. It has been demonstrated that breast-fed infants born to vitamin D-replete mothers had depleted 25(OH)D concentrations by eight weeks post-delivery.[160] Further, an Alaskan study indicated that breast-fed children were more likely to have deficient vitamin D levels with a relative risk of 3.6 over those fed formula.[184] This suggests that the limited amount of vitamin D found in breast milk is insufficient to provide adequately for the newborn.

Human milk contains little vitamin D.[21] The main form of vitamin D in human milk is 25(OH)D, which, in unsupplemented mothers, has been found to be in the range of 20–70 IU/L.[185–189] As expected, maternal sun exposure and vitamin D supplementation influences lactation levels of vitamin D, as does race.[181,190,191] Specker *et al.* found African-American women to have lower vitamin D milk status than white women.[192] This is likely due, at least in part, to the differences in cutaneous synthesis of vitamin D as darker skin pigmentation requires longer sun exposure to produce comparable serum 25(OH)D compared to lighter skin pigmentation. Maternal vitamin D status during pregnancy and infant sun exposure have a greater influence on vitamin D status of the infant than does maternal vitamin D status during lactation.[192]

Several groups have examined vitamin D supplementation during lactation (Table 5). Ala-Houhala *et al.* demonstrated seasonal variation in 25(OH)D levels of human milk and found that women in Finland had higher 25(OH)D concentrations in September than in February even when supplemented with 25 μg (1,000 IU)/day of vitamin D.[193] This suggests two possibilities: 1) that 25 μg/day is insufficient to reach a plateau or maintain 25(OH)D concentrations, since seasonal variation is still evident, and 2) that sunlight exposure is the primary influence on vitamin D content in mothers and their breast milk. The same group also showed that supplementation with 25 μg (1,000 IU)/day of vitamin D only minimally increased 25(OH)D

TABLE 5 Summary of Vitamin D Supplementation Studies During Pregnancy

Reference	Location & Latitude	Population	Vitamin D Protocol	25(OH)D (nmol/L)		Other outcomes
				Delivery	Endpoint	
Ala-Houhala[169]	Tampere, Finland 61.5°N	Mother-infant pairs	20-wk supplementation during breastfeeding	**Summer**		No significant intergroup differences in Ca, P, Mg or alk phos.
		Summer: Jul–Dec (n = 45)	I: mothers 1,000 IU/day	Mothers:		Antirachitic activity in breast milk of moms with 1,000 IU/day is insufficient in winter.
		Winter: Jan–May (n = 47)	II: infants 400 IU/day	I (n = 15): ~50	~ 42.5	
			III: infants 1,000 IU/day	II (n = 16): ~42.5	~ 32.5	
			Randomized trial	III (n = 14): ~50	~ 37.5	
				Infants:		
				I (n = 15): ~42.5	~ 30	
				II (n = 16): ~37.5	~ 100	
				III (n = 14): ~32.5	~ 132.5	
				Winter		
				Mothers:		
				I (n = 17): ~22.5	~ 62.5	
				II (n = 15): ~22.5	~ 50	
				III (n = 15): ~22.5	~ 37.5	
				Infants:		
				I (n = 17): ~25	~ 30	
				II (n = 15): ~20	~ 80	
				III (n = 15): ~25	~ 110	

TABLE 5 Summary of Vitamin D Supplementation Studies During Pregnancy *(Continued)*

Reference	Location & Latitude	Population	Vitamin D Protocol	25(OH)D (nmol/L)		Other outcomes
				Delivery	Endpoint	
Ala-Houhala[194]	Tampere, Finland 61.5°N	Mother-infant pairs (n = 49)	15-wk supplementation during breastfeeding	Winter (Jan–Apr):		No significant intergroup differences in maternal or infant Ca, P, albumin, PTH, or ALP.
				Mothers:		
			I (n = 17): mothers 2,000 IU/day	I: ~27.5	~ 90	
			II (n = 16): mothers 1,000 IU/day	II: ~25	~ 75	
			III (n = 16): infants 400 IU/day	III: ~25	~ 30	
			Randomized trial	Infants:		
				I: ~22.5	~ 72.5	
				II: ~17.5	~ 37.5	
				III: ~20	~ 80	
Greer[195]	Cincinnati, USA 39°N	Mother-infant pairs (n = 18)	12-wk breastfed only	Infants:		Significantly higher BMC in D+ versus placebo.
			I: placebo (n = 9)	I: ~50	~ 50	
			I: infants 400 IU/day (n = 9)	II: ~72.5	~ 97.5	
			Randomized double-blinded trial			
Rothberg[199]	Johannesburg, South Africa 26°S	Caucasian mother-infant pairs (n = 77)	6-wk supplementation	Mothers:		No intergroup differences Ca, P, or ALP.
			I: mothers placebo (n = 20)	30.0	I (n = 10): 25	Infant 25(OH)D was unaffected by with 500 or 1,000 IU/day maternal supplementation.
			II: mothers 500 IU/d (n = 20)		II (n = 9): 34.5	400 IU/d directly sig. raised infant 25(OH)D.
			III: mothers 1,000 IU/d (n = 20)		III (n = 9): 36.8	
			IV: infants 400 IU/d (n = 17)		IV (n = 12): 27.5	
			Randomized double-blinded	Infants:		
				22.3	I (n = 10): 2.8	
					II (n = 9): 25.5	
					III (n = 9): 23.5	
					IV (n = 12): 38.0	

in nursing infants[169] and that supplementing the infants directly was more effective in raising their 25(OH)D concentrations.[194] Further, breast-fed infants not receiving vitamin D supplements had decreased 25(OH)D concentrations during the winter, whereas infants who had received 10 μg (400 IU)/day of vitamin D maintained starting 25(OH)D concentrations.[195] Greer and Marshall found that white infants who were exclusively breast-fed during the winter months in Wisconsin maintained minimal vitamin D status over a six-month period with steadily declining 25(OH)D levels.[196] Recently, Hollis *et al.* supplemented mothers with either 40 μg (1,600 IU)/day of vitamin D2 or 60 μg (3,600 IU)/day of vitamin D2, each in combination with 10 μg (400 IU)/day of vitamin D3 for three months.[197] The increase of vitamin D seen in breast milk was 34.2 IU/L and 94.2 IU/L, respectively. The amount of 25(OH)D2 in the infants was found to reflect the maternal intake as well as the amount contained in the milk. We can infer from the above studies that a maternal intake of 10 μg (400 IU)/day of vitamin D does not sufficiently elevate the vitamin D status of mothers or nursing infants. A maternal supplementation of 50 μg (2,000 IU)/day increases maternal 25(OH)D concentrations, but the amount that passes into breast milk is insufficient to raise infant 25(OH)D concentrations. Breast milk 25(OH)D levels have been found to parallel those in the mother's circulation, but this is not reflected in the vitamin D status of the infant unless the mother is consuming high doses of supplemental vitamin D.[198] Therefore, the amount of maternal vitamin D supplementation required for adequate vitamin D status for both mother and infant is likely to be >50 μg (2,000 IU)/day. Directly supplementing the infant may prove to be more efficacious.[199]

Bone mineralization is of importance during the first years of life as this is a period of rapid development. There have been several investigations in preterm and term infants of different feeding regimens with an outcome comparing changes in BMD or BMC at both peripheral body sites and total body BMC.[196,200–205] Breastfed infants have lower bone accretion compared with formula-fed infants. The low vitamin D content of human milk is thought to contribute to the lower bone accretion observed in breast-fed infants.[192] Vitamin D supplementation is required even in breastfed infants.

F. Vitamin D Supplementation in the Infant: Impact on Skeletal Parameters

Randomized controlled trials examining the effect of increased 25(OH)D concentrations on bone health outcomes in infants are inconsistent. Infants given 10 μg (400 IU)/day of vitamin D2 for six months attained 25(OH)D concentrations of 92 nmol/L (n = 22) vs. 59 nmol/L in the placebo group (n = 24), with no difference between groups in bone mineral content.[196] Six infants fed (400 IU)/day of vitamin D2 had significantly higher 25(OH)D concentrations vs. placebo (n = 7), 81 and 32 nmol/L,

respectively.[206] A transient increase in BMC at 12 weeks was seen in the supplemented group, but by 26 weeks there was no difference between the groups with respect to BMC.[206] Compliance was reported to be 80%. The lack of difference may be due to the short duration, small sample size, ineffective dose, or simply because there is no benefit to be derived.

Zamora *et al.* demonstrated retrospectively that infants who had received ≥10 μg (400 IU)/day of vitamin D had significantly higher BMC and BMD at the femoral neck and greater trochanter at eight years of age than children who had not received any vitamin D supplementation.[207] Thus, vitamin D status during infancy, being an essential factor for osteoid mineralization, is likely to play a role in childhood bone-mineral accrual with consequences seemingly extending into adulthood. In Finland, from the mid-1950s until 1964, the recommended intake of vitamin D for infants was 100–125 μg (4,000–5,000 IU)/day.[208] There were no reports of idiopathic infantile hypercalcemia or any other health problem ever described under this supplementation regimen,[208] demonstrating the safety of intakes as high as 125 μg (5,000 IU)/day.

Poor vitamin D status during pregnancy and in infancy may thus result in reduced bone mineralization as well as an increased risk of osteoporotic fracture later in life.

G. Does Vitamin D Status Relate to Infant Birth Weight and Brain Development?

Vitamin D status is clearly related to season, and there are many relationships between season and physical and health measures. Birth weight is associated with a wide range of cognitive and behavioral outcomes and may influence brain development. There is seasonal fluctuation in birth weights within the normal range; furthermore, superior neurocognitive outcomes have been associated with heavier birth weights.[209–213] A report from India shows that intelligence and academic performance at age 12 years of age was significantly lower in children with low birth weight (LBW) in comparison with those of normal birth weight (NBW).[214] Similarly, 16-year-old Chinese children who at birth were small for gestational age (SGA) or LBW (1,200–2,500 g) were found to lag behind NBW peers in physical growth, cognitive capacity, and school achievement.[215] Infant birth weight may be associated with cognitive development and physical health.

Fetal responses during gestation are determined by the maternal environment, health, and physiology. Annual periodicity of birth weight, with the heaviest births occurring in October and the lightest in May, has been shown,[216] which reflects the known seasonal variation in 25(OH)D levels. Infants born during the summer months, when 25(OH)D levels are at their nadir, had lower birth weights when compared with infants born in spring-winter months,[217–220] when 25(OH)D concentrations are highest. Similarly,

mean length of newborns in Tehran was found to be significantly higher in newborns whose mothers had adequate calcium and vitamin D intake (n = 212) when compared with those whose mothers had inadequate intake (n = 237). Further, the incidence of LBW was significantly lower in the adequate maternal intake group when compared with those in the inadequate maternal intake group.[221] Not only have 25(OH)D concentrations been associated with birth weight but cord 1,25(OH)2D levels have been shown to be lower in SGA than NBW infants,[222] suggesting a link between 1,25(OH)2D action and growth.

This association may be explained at a molecular level. At latitudes >50°, the winter season has lower levels of UVB radiation and consequently lower levels of vitamin D3 biosynthesis. In rat brains and spinal cords, oligodendrocytes have been shown to express VDR and are sensitive to vitamin D.[223] The presence of 1α-OHase, VDR, and 24-OHase in the brain[224] suggests that cells can locally synthesize, utilize, and subsequently degrade 1,25(OH)2D as required. *In vitro,* microglial cells can produce 1,25(OH)2D from 25(OH)D.[225] VDR expression in the rat brain is altered during brain development.[226] This prompts speculation that vitamin D may play a role in brain development. Eyles *et al.* have demonstrated in a rat model that low prenatal vitamin D3 could impair brain development.[227] Thus, the seasonality of birth weight demonstrated by the above studies coupled with a known seasonal variation in vitamin D status may indicate a link between vitamin D3 status and birth weight, which may also have effects on skeletal, neurological, and cognitive development of the fetus.

IV. THE YEARS BETWEEN INFANCY AND PUBERTY

Data pertaining to bone mineralization and vitamin D nutrition during prepubertal years are scarce; however, it is possible to glimpse the vitamin D nutritional status for some groups (Table 6). In Spanish children (8 ± 2 years) of age the mean 25(OH)D concentration was 73 ± 24 nmol/L in summer, and 38 ± 13 nmol/L in winter.[228] Eighty percent had 25(OH)D concentrations <50 nmol/L during winter months.[228] In Argentinean children (8.5 years, of age n = 42) mean 25(OH)D concentrations were 45 nmol/L at the end of summer and 24 nmol/L at the end of winter—insufficient at both times.[18] Finnish children (6–10 years) demonstrated seasonal variation in 25(OH)D concentrations, and 16.8% were severely deficient (<12.5 nmol/L) in winter.[229] In 10–12-year-old Finnish girls (n = 193), 78% had 25(OH)D concentrations <40 nmol/L.[230] Adequate vitamin D status has been seen in some regions, mostly of a sunny clime, with mean 25(OH)D concentrations ranging from 80–100 nmol/L.[25–27]

Significant seasonal variation occurs in growth measures, height velocity, and lower-leg-length velocity.[231] Since vitamin D is known to assist in skeletal

TABLE 6 Observational Studies in Prepubertal Children and Adolescents Assessing the Dietary Intake of Calcium and Vitamin D and the Prevalence of Vitamin D Insufficiency

Reference	Population & Age (y)	Location & Latitude	Dietary Intake		Serum 25(OH) (nmol/L)		D% Vitamin D	
			Calcium (mg/day)	Vitamin D (μg/day)			Deficient	Insufficient
Marwaha[350]	5,137 children 10–18 y	New Delhi, India 28°N		NA	29.5 ± 18.0		35.7[c]	92.6[f]
								84.9[f]
El-Hajj Fuleihan[495]	363 girls & boys 10–16 y	Beirut, Lebanon 33.4°N	710 ± 382	3.75 ± 3.98	Spring:	Fall:	Spring:	
					42.5 ± 20.0	55.0 ± 17.5	44[c]	36[f]
Fares[263]	92 girls & 80 boys 10–17 y	Beirut, Lebanon 33.5°N	NA	NA	Girls:	Boys:		40[e]
					47.5 ± 17.5	60.0 ± 15.0		
Salamoun[496]	207 girls + 178 boys 10–16 y	Beirut, Lebanon 33.5°N	842 [786, 897]	3.63 [0.63, 4.15]	NA		16% met AI for vitamin D	
Willis[234]	96 girls 4–8 y	Georgia, USA 34°N	499.6 ± 154.9	5.1 ± 3.3	Summer:	Winter:	18%[f]	
				black:	72.0	49.0		
				white:	102.0	83.5		
Looker[497]	699 girls & 625 boys 12–19 y	NHANES II, USA	NR		Summer	Winter		
					(25–47°N):	(25–41°N):		
				boys:	89.5	78.6	12[e]	13[f]
				girls:	80.5	64.9	5[e]	13[f]
Du[498]	1,248 girls 12–14 y (12.9 ± 0.5)	Beijing, China 40°N	Urban :		Summer:	Winter:	45.2[f]	NR
			396 ± 91	1.55 ± 1.03	30.2 ± 11.9	13.9 ± 9.6		
			Suburban:					
			352 ± 87	0.75 ± 1.03	24.7 ± 10.6	12.7 ± 5.9		
			Rural:					
			318 ± 94	0.59 ± 0.65	23.8 ± 8.7	12.8 ± 6.7		

Zhu[499]	698 girls 10.1 ± 0.4 y	Beijing, China 40°N	431 ± 167	0.97 ± 0.76	19.2 ± 8.0		NR	NR
Lapatsanis[500]	178 children 3–18 y	Northwestern Greece ~40°	NR	Age range	Summer:	Winter:		
				3–10 y:	73.4 ± 4.5	46.2 ± 3.2	14[c]	15[f]
				11–14 y:	66.4 ± 3.5	52.4 ± 4.7	13[c]	9.4[f]
				15–18 y:	69.1 ± 3.2	31.7 ± 2.5	47[c]	21[f]
Taha[348]	32 boys, 20 girls 15–19 y	Brooklyn, USA 40.6°N (Jewish population)	908±506	7.2 ± 4.3	46.0 ± 19.0		NR	NR
Harkness[501]	370 girls 15.5 ± 1.6y	Cleveland, USA 41°N	NR	53.7 (50.7–56.7)			17[d]	54[f]
				African-American girls: 43.0 (39.8–46.3) (n = 234)			26[d]	71[f]
Gordon[502]	307 girls & boys 11–18 y	Boston, USA 42°N	NR		Summer:	Winter:	4.6[c]	42[f]
					~ 45	~ 62		
Sullivan[503]	23 girls 9–11 y	Bangor, USA 44°N	1008 ± 323	5.12 ± 2.16	Spring:	Fall:	NR	48[f]
				1st y (n = 22):	74.4	55.9		
				2nd y (n = 22):	70.8	53.9		
				3rd y (n = 20):	72.3	50.0		
Ginty[256]	92 boys, 104 girls 11–16 y	Canton de Vaud, Switzerland 46.5°N	60–70% met AI	30–60% met AI	Girls:	Boys:	NR	16[d]
					42.3 ± 12.3	42.5 ± 11.8		
Guillemant[319]	28 boys 13–16 y	Gouvieux, France 49°N	NA	NA	Spring:	Fall:		
					16.5 ± 5.1	74.8 ± 18.6	50[b]	NR
Ellis[504]	West Indian boys (n = 67)	Birmingham, UK 52°N	NA	1.51 (0.4–3.8)	22.2 ± 8.7		NR	NR
	Asian boys (n = 124) 13 y				12.7 ± 7.0			

TABLE 6 Observational Studies in Prepubertal Children and Adolescents Assessing the Dietary Intake of Calcium and Vitamin D and the Prevalence of Vitamin D Insufficiency *(Continued)*

Reference	Population & Age (y)	Location & Latitude	Dietary Intake		Serum 25(OH) (nmol/L)		D% Vitamin D	
			Calcium (mg/day)	Vitamin D (μg/day)			Deficient	Insufficient
Das[505]	i) 14 white girls 15.2 y (14.8–16.6)	Manchester, England 53°N	NA		1.2 (0.3–5.6)	37.3 (18.3–73.3)	17[f]	73[d]
	ii) 37 non-white girls 15.3 y (14.7–16.3)		NA		1.4 (0.4–6.0)	14.8 (5.8–42.8)		
Hill[506]	22 girls 11–13 y	Cork, Ireland~52°N	NA		NR	NR	Spring:	
			NA				NA	9[f]
							Winter:	
							NA	85[f]
Hill[507]	457 boys 455 girls 12 or 15 y	Northern Ireland ~54°N	907 (390)	1.21 [1.12, 1.31]	NR	NR	Summer:	
							NA	8[f]
							Winter:	
							NA	52[f]
Lotborn[508]	396 children 15 y	Trollhattan (58.3°N) & Uppsala (59.9°N), Sweden	1049 ± 298	4 ± 2	NA	NA	NA	

Andersen[342]	199 girls 12.6 ± 0.5 y	i) Denmark, 56°N (n = 59)	8.3 [2.6,24.8]	2.4 [0.9,4.9]		24.4 [9.4,56.7]	51[c]	93[f]
		ii) Finland, 64°N (n = 60)	10.9 [5.5,24.5]	5 [1.2,12.2]		29.2 [12.6,53.5]	37[c]	97[f]
		iii) Ireland, 53°N 7.3 (n = 19)	7.3 [5.4,22.6]	2.4 [1.2,7.5]		41.3 [18.6,59.3]	26[c]	89[f]
		Poland, 52°N (n = 61)	5.2 [1.2,15.8]	3.1 [1.2,8]		30.6 [15.2,62.4]	33[c]	87[f]
Outila[349]	178 girls 14–16 y	Helsinki, Finland 60°N	1216 ± 591	4.3 ± 2.8		39.0 ± 14.0	13.45[c]	61.8[e]
Lamber-Allardt[509]	875 boys 893 girls 3–18 y	Finland~60°N	NA	3.9 ± 5.7		NA		
				3.5 ± 6.2				
Ala-Houhala[229]	284 children 2–17y	Finland (61–66°N)		NA	Summer:	Winter:	Winter:	
					67.9 ± 25.7	33.2 ± 25.5	2–5 y:	7.5[a]
							6–10 y:	16.8[a]
							11–17 y:	23.5[a]
Cheng[230]	193 girls 10–12 y	Jyväskylä, Finland 62°N	733 ± 274	2.7 ± 1.7		NR	32[c]	46[e]
Lyytikainen[510]	860 girls 10–12 y	Jyväskylä, Finland 62°N	1117 ± 420	3.1 ± 1.9		NA	12% met AI	
Jones[511]	136 boys 16.7 ± 0.8y	Burnie, Tasmania 41°S	NA			43.6 ± 15.3	11[c]	68[f]

NA/R = Not assessed/reported.
Deficiency/Insufficiency defined as 25(OH)D (nmol/L) <12.5[a] ;<15[b]; <25[c];<30[d]; <40[e]; <50[f]
Weighted averages are presented for articles that divided subjects' 25(OH)D concentrations into groups.
[ ] denotes 95% CI; ± denotes SD.

growth, it seems likely that seasonal variations in growth rates may be related to the well-documented seasonal variations in vitamin D nutritional status. In support, vitamin D intake was found to be the most important predictor of medullary diameter in a study of premenarcheal girls aged 9–11 years (n = 373).[232] Finnish girls (10–12 years of age, n = 193) with 25(OH)D concentrations <25 nmol/L (n = 61) had significantly higher tartrate resistant alkaline phosphatase and PTH concentrations and lower femoral BMC than did girls with 25(OH)D levels >40 nmol/L (n = 43).[230] Further, baseline 25(OH)D concentrations in 171 Finnish girls (9–15 years of age) correlated with three-year change in BMD at the lumbar spine and femoral neck, with greater BMD increases in girls with higher 25(OH)D status.[233] The associations found between vitamin D intake and measures of skeletal growth suggest that vitamin D status has an influence on bone-mineral accretion.

A decline in vitamin D status from 4–8 years of age was reported in a group of girls from Georgia, USA (n = 96).[234] After adjustment for fat-free soft tissue mass, there was no longer a relationship between 25(OH)D and age, suggesting that 25(OH)D has a role in the acquisition of lean mass with growth. Dietary intake was 5.1 μg (204 IU)/day (25(OH)D concentrations found in Table 6).[234] Age-dependent changes in 1,25(OH)2D concentrations have also been reported, and 1,25(OH)2D concentrations are higher in children when compared with adult levels.[235] Serum 1,25(OH)2D concentrations appear to increase with age from childhood into adolescence likely due to the demand of the growing skeleton and possibly to increases in muscle mass.

In an open-label, one-month study of 10 μg (400 IU)/day vitamin D supplementation in 6–10-years-old African-American children (n = 41), no difference in 25(OH)D concentrations or bone markers was found.[236] The mean dietary intake of vitamin D for these children was 6.9 μg (277 IU)/day.[236] In a randomized, double-blinded, placebo-controlled trial, 24 8–10-years-old children were supplemented with vitamin D at 10 μg (400 IU)/day for 13 months.[237] Levels of 25(OH)D at six and 12 months were found to be significantly higher in the supplemented group; however, seasonal variation in 25(OH)D levels was still evident, suggesting that a higher vitamin D intake is required to stabilize 25(OH)D concentrations. Supplementation with vitamin D produced no effect on calcium measures, nor was a difference detected between those who received the vitamin D and those who received placebo (n = 27) in measures of BMC or growth, yet this is likely due to an inadequate dose. The available data suggest that increased awareness of vitamin D status is warranted in this age group, and further research is required regarding supplementation.

TABLE 7 Vitamin D Supplementation Studies in Prepubertal Children and Adolescents

Reference	Location & Latitude	Population & Age	Vitamin D Protocol	25(OH)D (nmol/L)		Other outcomes
				Baseline	Endpoint	
El-Hajj Fuleihan[341]	Beirut, Lebanon 33.5°N	179 girls 10–17 y	Double-blinded randomized			aBMC and total hip BMC sig higher in D+H group.
			D+L: 5 μg/d D3	35.0 ± 22.5	42.5 ± 15.0	Sig increase in lean mass in both D+ groups vs D.
			D+H: 50 μg/d D3	35.0 ± 20.0	95.0 ± 77.5	No change in Ca levels in D+.
			D-: placebo	35.0 ± 17.5	40.0 ± 20.0	
Rajakumar[236]	Pittsburgh, USA 40°N	42 African Americans 6–10 y	10 μg/d vitamin D2 for 1 mo	60.0 ± 26.2	68.6 ± 18.7	49% had 25(OH)D <50 nmol/L at baseline vs 18% at endpoint.
Docio[228]	Cantabria, Spain 43°N	21 children 9 ± 1 y	40 μg/d 25(OH)D for 7 d in Mar or Oct	~ 70	~ 86	No change in calcium. 1,25(OH)2D sig increase, PTH sig decrease. Suggest 50 nmol/L desirable 25(OH)D.
Guillemant[358]	Gouvieux, France 49°N	54 boys 15 y ± 10 mo	Paired for height, weight, & Tanner stage, ea. pair randomized	September:	March:	78% of D- 25(OH)D <25 nmol/L.
			D+: 2.5 mg every 2 mo (Sep, Nov, Jan) ≈4.2 μg/d	D+: 61.0 ± 15.5	55.2 ± 11.5	iPTH sig higher in D- in Sept vs Mar, D+ iPTH unchanged.
			D−: no supplementation	D−: 53.7 ± 12.2	20.2 ± 0.5	
Schou[512]	Denmark 56°N	20 children 9.8 y (6.2–13.7)	Double-blind randomized	D+ then D−:		PTH sig reduced.
			D+: 15 μg/d vitamin D for 4 wk, 2 wk wash-out, cross-over	32.3 ± 4.1	43.4 ± 2.9	No variation in markers of bone turnover or difference in lower-leg growth rates.
			D−: placebo	D− then D+: 33.7 ± 3.3	50.2 ± 4.5	

TABLE 7 Vitamin D Supplementation Studies in Prepubertal Children and Adolescents *(Continued)*

Reference	Location & Latitude	Population & Age	Vitamin D Protocol	25(OH)D (nmol/L)		Other outcomes
				Baseline	Endpoint	
Lehtonen-Veromaa[317]	Turku, Finland 60°N	191 girls 14–16 y	10 μg/d vitamin D2 for 3 mo (Oct–Jan)	Summer:	Winter:	67.7% had 25(OH)D <37.5 nmol/L.
				62.9 ± 15.0	33.9 ± 13.9	Supplementation insufficient to prevent hypovitaminosis D.
Lehtonen-Veromaa[233,351]	Turku, Finland 60°N	171 girls 9–15 y	1st year: 10 μg/d D2 Oct–Jan	31.3 ± 11.6	1st y: 33.3 ± 11.1	20 μg/d D2 adequate to raise winter 25(OH)D concentrations.
			2nd year: 10 μg/d D2 Oct–Feb		2nd y: 33.8 ± 13.7	Significant association between baseline 25(OH)D and 3y ΔBMD and ΔBMAD at lumbar spine & femoral neck.
			3rd year: 20 μg/d D2 Oct–Mar		3rd y: 40.6 ± 15.8	
Viljakainen[354]	Helsinki, Finland 60°N	228 girls 11.4 ± 0.4 y	Stratified randomization			Significantly higher BMC in 10 μg/d group—17.2% higher than placebo group, 5 μg/d group 14.3% higher BMC vs placebo.
			D: placebo (n = 73)	D-: 47.8 ± 18.2	42.8 ± 30.1	
			D5: 5 μg/d vitamin D3	D5: 46.3 ± 17.4	51.7 ± 32.7	
			D10: 10 μg/d vitamin D3	D10:46.7 ± 16.2	58.8 ± 29.7	
			1 supplementation			

Ala-Houhala[237]	Tampere, Finland 61°N	66 children 8–10 y	Double-blind randomized			Seasonal variation present in D+ & D-groups.
			D+: 10 μg/d vitamin D (n = 24)	49.5 ± 19.0	71.1 ± 23.7	No change in Ca, Alb, Pi, alk phos, PTH, BMC, or growth.
			D−: placebo (n = 27)	45.9 ± 15.5	43.1 ± 19.5	
			13-mo supplementation			
Cheng[513]	Jyväskylä, Finland 62°N	146 girls 11.0 ± 0.7	2 y double-blind randomized	NR	NR	No overall differences in markers of bone turnoverbody weight, height, BMC, or aBMD.
			CaD: 1000 mg/d Ca + 5 μg/d			
			Ca: 1000 mg/d Ca + D placebo			
			P: Ca + D placebo			

V. ADOLESCENCE

A. Bone Development in Adolescence

Normally, adolescent development is characterized by rapid growth in height and weight. Puberty is accompanied by changes in body composition and hormones that induce primary and secondary sexual characteristics.[238] Overall, the length and mass of the body grows as the skeleton grows within. Bone undergoes a continuous process of formation and resorption; during growth, especially in adolescence, the velocity of bone formation is predominant.[238]

The bone is a very active tissue, undergoing formation until the end of growth in length when full pubertal development is achieved, which for girls is between the ages of 13 and 16 years and for boys between 15 and 18 years.[239] During bone growth, osteoblasts synthesize the bone matrix, which is primarily comprised of type 1 collagen. 1,25(OH)2D can directly influence bone growth as it can alter osteoblasts as they undergo differentiation from early proliferating cells to mature osteoblasts.[240] 1,25(OH)2D is also required to sustain a calcium concentration suitable for mineralization of the osteoid. Without 1,25(OH)2D, rickets and/or osteomalacia will result.

Throughout life, bone is continuously formed and resorbed. Osteoclasts resorb bone primarily by PTH stimulation. At excessive concentrations, vitamin D and its metabolites can also stimulate bone resorption.[81,241] During skeletal growth, osteoblastic activity exceeds osteoclastic.[238] Together, osteoblasts and osteoclasts balance bone modeling and remodeling, for acquisition and maintenance of bone mass.

Peak bone density and maximum growth velocity do not occur at the same time, particularly in girls, in whom peak bone density is achieved at a later time than maximal growth velocity.[242] BMD peaks before 20 years of age, whereas skeletal mass peaks 6–10 years after this.[242] Puberty is an important time for bone mass acquisition. Between the onset of puberty and young adulthood, skeletal mass approximately doubles,[243] and roughly 37% of an adult's total bone mass is accumulated during this time.[244–246] Ilich *et al.*[247] have shown that up to 10% of an adult's peak bone mass (PBM) may be accumulated in a single year during the pubertal growth spurt. It follows that such rapid skeletal growth requires optimal mineral intake for utilization during bone building.

During puberty, when rapid bone growth is occurring, concentrations of osteocalcin increase dramatically.[248,249] Osteocalcin is a non-collagenous, bone-specific protein released by the osteoblast into the circulation in proportion to the rate of bone formation. Synthesis of osteocalcin by the osteoblast is stimulated by the action of 1,25(OH)2D.[250] BMD is one of the main determinants of a bone's ability to withstand loading,[251] and it is related to bone formation markers, whereas bone resorption markers relate to

bone volume and skeletal size. Some have found the highest concentrations of bone formation markers to occur in the early stages of puberty,[252,253] others at mid-puberty,[254,255] and still others during the later stages of puberty.[256] Overall, in both boys and girls, the maximal accumulation of BMD at the lumbar spine and femoral neck occurs after peaks in formation markers during the last stages of puberty.[257–262]

The greatest increase in bone mass occurs between the ages of 12 and 15 years in girls and between 14 and 17 years of age in boys, after which the rate of bone mass accrual declines ages (16–18 years and 17–20 years, respectively).[239] In girls, menarche occurs near the end of the pubertal growth spurt and is accompanied by rapid increases in bone mass and BMD. Increases in BMD occur in combination with peak concentration of bone formation markers, as demonstrated by increased procollagen I aminopropeptide (PINP) and c-terminal of type I procollagen (CTx), peaking at Tanner stage 2–3 in girls and stage 3–4 in boys.[256,263] Increases in bone formation and mass occur in tandem with the peak height velocity. Other type I collagen telopeptide markers of bone resorption peak in concentration during this time period, namely N-telopeptide of type I collagen (NTx), ICTP, and carboxy-propeptide (PICP).[253,264,265] Though height gain decreases during the last stages of puberty, bone mass accumulation, at a decreased rate, still occurs after the peak height velocity in both sexes.[266]

Maximal bone growth during puberty is induced by the synergistic actions of growth hormone (GH), insulin-like growth factor-1 (IGF-1), sex steroid hormones, and nutritional factors.[267] The sex steroid:GH:IGF-I axis has a major role in skeletal maturation, pubertal growth of bone, and muscle mass accrual.[268,269] GH induces the acceleration of bone turnover and stimulates osteoblast proliferation and formation of new bone.[270] GH, IGF-I, IGF-II, and IGF binding proteins (IGFBPs) control growth, remodeling, and mineralization of the skeleton, in part via direct action on bone.[271] GH plays an important role in bone growth as well as in the development of bone mass and density.[272] GH drives the skeletal IGF-I synthesis; IGF-I enhances hypothalamic outflow, promotes sex hormone secretion,[271] and promotes longitudinal bone growth and cortical and trabecular bone formation.[273] GH and IGF-I significantly increase renal 1-αOHase activity and serum 1,25(OH)2D concentrations.[274–276]

Sex steroids, IGF-II, and glucocorticoids modulate the secretion of GH and IGF-I.[277] IGF-I is an indirect modulator of osteoblastic activity [278] and has a positive association with growth rate acceleration.[279] 1,25(OH)2D, through the VDR, down-regulates IGF expression.[280] Matileinen *et al.* have demonstrated that IGFBPs 1, 3, and 5 are primary target genes of 1,25(OH)2D.[281] This indicates that 1,25(OH)2D may have some regulatory control over this system, further emphasizing the importance of vitamin D in skeletal growth and maturation. During puberty, GH and IGF-I increase dramatically, augmented by the increasing levels of sex steroids.[282]

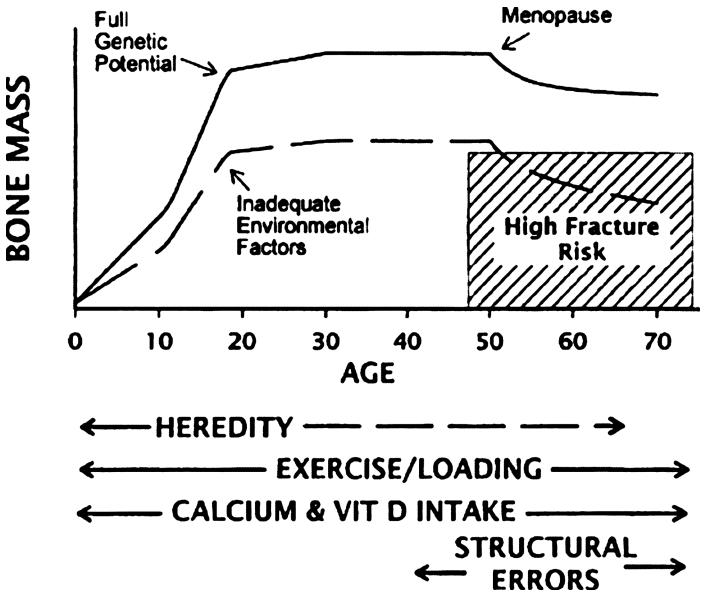

FIGURE 3 Diagrammatic representation of the life-line of bone mass in individuals who do or do not achieve their full genetic potential for skeletal mass (from Ref. 283, used with permission)

Genetics play a predominant role in achieving PBM, and polymorphisms of GH, IGF, IGFBP, sex steroid receptors, VDR, and skeletally active cytokines are among the many factors that contribute to this. Heaney *et al.* have reviewed the various factors contributing to the achievement of PBM.[283] Figure 3 illustrates the importance of achieving the "full genetic potential," defined as a skeleton size and mass that have not been restricted by an insufficient supply of nutrients and/or suboptimal weight-bearing exercise, as it can protect against fragility fractures later in life.

Increased risk of fracture is related to BMD. Low BMD values are more common in the skeletons of girls who have had forearm fractures than in girls who had never broken a bone.[284–286] Boys (3–19 years of age, n = 100) have the greatest incidence of fractures (57%) between the ages of 11 and 15 years, with a peak occurrence at 13 years of age. Overall, in adolescence the most common fracture occurs at the distal radius,[287–291] and, compared with prepubertal adolescents or young adults, the annual incidence of fracture is highest in both girls and boys from the ages of 10 to 15 years.[291,292] This increased incidence of fracture is concurrent with peak height velocity and is likely associated with a reduced BMD during rapid growth.[293] Indeed, several investigations have demonstrated significantly lower BMD values in children who have experienced fractures when compared with those who have not.[284,291,294] Girls with a low BMC at baseline who had forearm fractures did not have greater BMC at follow-up at any site (total body, lumbar spine, radius, and hip trochanter),[291] indicating that low BMC is not compensated for

during skeletal growth and persists into young adulthood. Further, reduced remodeling activity in adolescence is associated with reduced BMD.[295] Bone mineralization depends on adequate supplies of 1,25(OH)2D, calcium and phosphorus. A deficiency of any of these factors can result in defective bone mineralization. Vitamin D deficiency in particular results in higher PTH levels, which can lead to even higher levels of bone remodeling.[263] Restrictive 25(OH)D concentrations during the pubertal growth spurt may therefore be deleterious to bone mass accretion. In support, a positive correlation between bone mass and sun exposure has been reported in prepubertal children.[296] Unlimited vitamin D production has a positive effect on bone growth.

Osteoporosis occurs in all populations at all ages (NIH consensus statement 2000) and has significant physical, social, and economic consequences. Osteoporosis was originally characterized as a pediatric disorder that manifested itself in old age,[297] with its roots starting in fetal life.[163] Indeed, maternal circulating 25(OH)D concentrations during the third trimester have been correlated with skeletal size of the offspring at birth and one year[164] and with offspring pre-pubertal BMD at nine years.[165] Risk for osteoporosis is reflected by low BMD, with PBM being a major factor influencing the risk of osteoporotic fractures later in life. Fracture rates in children, like adults, are higher in individuals with lower BMD values.[285,286,294,298] The optimal PBM required to prevent osteoporotic fractures later in life is not known, yet reaching one's potential PBM is certain to have an impact. Adequate calcium and vitamin D intake is crucial to achievement of optimal PBM and to preservation of bone mass throughout life.

B. Effects of Vitamin D on Bone Development

Calcium requirements are increased during adolescence. Sufficient 1,25(OH)2D is critical for calcium absorption and retention, which are highest during rapid skeletal modeling in infancy and adolescence. An increased 1,25(OH)2D requirement is needed to meet the demand for calcium during these growth spurts.[247,299] The amount of dietary calcium consumed exerts a positive dose-dependent effect on the growing skeleton as shown by Recker *et al.*, where spinal bone mass in young women was positively correlated with calcium intake.[300] The efficiency of calcium absorption during puberty is increased according to bone formation, with maximal net calcium balance at intakes of 1200–1500 mg/day.[301] When calcium consumption is ample, from either dietary or supplemental intakes, the achievement of greater bone mass can be achieved.[302] In general, randomized trials with calcium or milk supplementation in youth have shown an increase in bone mass in adulthood.[303] If serum calcium and phosphorus concentrations are not adequate, mineralization of bone matrix is impaired. This is a characteristic of vitamin D deficiency in both children and adults. To meet the high calcium requirements of the growth spurt, adolescents must absorb calcium at higher

efficiency than prepubertal children or young adults.[299,304,305] Calcium absorption is mediated by the higher levels of 1,25(OH)2D in puberty,[247] and it is facilitated by 25(OH)D.[306]

The effects of vitamin D on bone development are not completely understood. It is well known that 1,25(OH)2D improves bone strength by stimulating intestinal calcium and phosphorus absorption.[307] Vitamin D is required for the optimal absorption of calcium, acting directly on the brush border of the intestine to increase calcium permeability. In adolescents, serum 25(OH)D concentrations above 62.5 nmol/L are required for optimal calcium absorption.[308]

The hormone 1,25(OH)2D has two effects on bone: it mobilizes calcium and phosphorus from the skeleton as required, and it promotes maturation and mineralization of the osteoid matrix. By affecting gene expression, 1,25(OH)2D alters matrix protein expression and synthesis, which, in turn, regulates mineralization. For example, osteoblastic synthesis of type I collagen, the predominant bone matrix protein, is regulated in part by 1,25(OH)2D. Together, the calciotropic hormones 1,25(OH)2D, PTH, and calcitonin maintain blood ionized calcium concentrations, within the remarkably narrow limits defined by the calcium environment, needed for neuromuscular function. The hormones act via the intestine, kidney, and skeleton. Expression of osteocalcin and osteopontin are up-regulated in osteoblasts by 1,25(OH)2D. Failure to mineralize the growth plate is a direct result of the calcium deficiency that causes rickets when vitamin D supplies are inadequate. Osteocalcin concentrations are increased during periods of rapid bone growth, reflecting the increased rate of bone formation.[248,249] Osteocalcin synthesis is regulated by 1,25(OH)2D; during puberty, serum 1,25(OH)2D and osteocalcin levels are closely related.[247] This indicates that 1,25(OH)2D is an important determinant of bone mass gain during this period of growth. Further, 1,25(OH)2D regulates calcium absorption and retention in the kidney to meet the needs for skeletal growth and mineralization.

PTH has a dual effect: at one end of the spectrum, when concentrations of PTH are within physiological ranges, PTH can stimulate bone anabolism; at the other end, increased concentrations of PTH can stimulate bone catabolism.[309] Decreased circulating concentrations of calcium increase serum PTH synthesis and secretion, which, in turn, stimulates the renal hydroxylation of 25(OH)D to 1,25(OH)2D. There is an inverse relationship between serum 25(OH)D and PTH, while PTH tends to stimulate 1,25(OH)2D production. Therefore, when 25(OH)D stores are adequate, PTH is suppressed, and bone mineral accretion can occur. The action of PTH is to stimulate calcium mobilization from bones. In support, Cheng *et al.* found that Finnish girls (10–12 years of age) with low serum 25(OH)D had corresponding high PTH levels and lower bone mineralization.[230]

Studies based on twins and mother-daughter pairs show that inheritance accounts for up to 70% of the variance of bone density in men and women.[310] Normal allelic variations in the *VDR* gene may play an important role in the intensity of the genetic effect on bone density in normal twins of both sexes.[311] Sainz *et al.* demonstrated that *VDR* gene alleles predict the density of femoral and vertebral bone in prepubertal American girls of Mexican descent.[312] Girls with *aa* or *bb* genotypes had increased femoral and vertebral bone density compared with girls of *AA* or *BB* genotypes.[312] Lebanese girls with the *bb* genotype had the largest increments in BMC in response to vitamin D at the lumbar spine, femur total body, and forearm at one year in the vitamin D trial (unpublished data). The same results were seen using the *Taq*I restriction enzyme to assess VDR polymorphisms (unpublished data). More studies are required to establish the full effect of vitamin D receptor genotypes on bone mass and bone mass accrual in the young.

Skeletal muscle is a target tissue of vitamin D.[313] Muscle mass reflects muscle strength and is linearly correlated with bone mass.[261,262,314,315] Muscle weakness, atrophy, and abnormalities in muscle contraction and relaxation are observed in vitamin D-deficiency states. These myopathies are independent of changes in blood mineral composition or PTH levels and respond only to vitamin D3 or its metabolites.[313] It has been demonstrated that 1,25(OH)2D is essential for normal homeostasis of intracellular calcium and stimulates proliferation and growth of skeletal muscle, and it also plays an important role in contractility. 25(OH)D may also play an independent role in phosphate uptake in myocytes and contractile protein synthesis. Vitamin D repletion prevents muscle weakness. Further, high-impact exercise has been demonstrated to have positive effects on the growing skeleton.[316] Building muscle has a positive effect on bone strength and mass. In addition to the effect of hormones and nutritional factors on bone mass, muscle mass and strength are important mechanisms for increasing bone strength and mass during puberty, another area where proper vitamin D nutrition comes into play.

Vitamin D insufficiency may contribute to poor bone health by decreasing calcium absorption and increasing PTH secretion, leading to increased bone turnover and bone loss. Vitamin D insufficiency results in undermineralized weaker bone that cannot optimally carry out normal functions. Vitamin D insufficiency has also been associated with muscle weakness, limb pain, and impaired muscle function. Further, vitamin D controls skeletal growth by increasing the expression, differentiation, and maturation of bone-building cells and factors. Vitamin D plays regulatory roles in the sex steroid:GH:IGF-I axis that has a predominant role in pubertal skeletal growth. It is therefore important to determine the optimal vitamin D status required for the acquisition of optimal peak bone mass and to determine at what age it becomes critical to maximize vitamin D and calcium intake. Some controlled trials

have indicated that supplementation of calcium before puberty is more effective, whereas others have found benefit in pubertal children.[283] There is, however, a lack of data concerning vitamin D supplementation during adolescence.

C. Is There an Increased Need for Vitamin D During Puberty?

During puberty the metabolism of 25(OH)D to 1,25(OH)2D may increase to meet growth demands for calcium absorption during the period of maximal height velocity. Evidence to support this is demonstrated by decrements in 25(OH)D concentrations found during periods of rapid growth [317,318] and increments in 1,25(OH)2D.[235,247,318–322] Hagg and Taranger found menarche to occur within the pubertal growth spurt; no girl experienced menstrual bleeding before peak height velocity, and all girls experienced menarche before the end of the spurt.[323] Prior to the onset of menarche (≥2 years before), 25(OH)D concentrations were found to be 10 nmol/L higher than 25(OH)D concentrations within two years of menarche.[318] The pubertal growth spurt occurs in conjunction with menarche in girls and may require higher 25(OH)D levels to meet an increased demand of 1,25(OH)2D for calcium absorption and possibly for muscle mass accrual. In parallel, Ginty *et al.* found that as Tanner stages in boys progressed, 25(OH)D levels declined, with the lowest 25(OH)D concentrations in boys at Tanner stage P4-5.[256] Guillemant *et al.* have demonstrated a negative relationship between 25(OH)D concentrations and the pubertal status of male adolescents.[319] Bonofiglio *et al.* demonstrated in a population of 200 girls (11–16 years of age) that BMD steadily increased as expected up to the age of 16 years, in addition to which post-menarcheal girls demonstrated a marked increase in serum 25(OH)D levels after the decrease seen during puberty.[324]

Aksnes and Aarskog showed that in girls between the ages of 11 and 12 years there is an increase in 1,25(OH)2D concentrations, while in boys the increase occurs from 13–14 years of age.[318] In both sexes the increase in 1,25(OH)2D coincides with an increase in bone formation as bone mass increases, in girls from 12–15 years of age and in boys from 14–17 years of age. Chesney *et al.* showed that children 13–16 years of age had significantly higher concentrations of 1,25(OH)2D than did prepubertal children (<11 years.[235] More recently, Guillemant *et al.* demonstrated a significantly positive correlation between 1,25(OH)2D and pubertal status.[319] In relation to stage of puberty, a maximal increase in 1,25(OH)2D was shown in girls between Tanner stages 1 and 2, peaking at stage 3, whereas in boys the increase occurred between stages 2 and 3,[318] also coinciding with the peak growth period.

Growth velocity correlated positively with 1,25(OH)2D concentrations.[320] Others have also demonstrated an increase in 1,25(OH)2D concentration associated with increases in age,[321] though still others have not.[322,325]

In a cross-sectional analysis of 178 healthy Caucasian girls (11.7 ± 2.3 years), Ilich *et al.* demonstrated that the greatest increase in total body BMD and BMC as well as radial BMD occurred between pubertal stages 3 and 4, which was associated with the highest concentrations of 1,25(OH)2D.[247] Further, they showed a significant positive association between 1,25(OH)2D concentrations and changes in various bone mass variables.

Bone formation markers increase during puberty. Lehtonen-Veromaa *et al.* found that increased levels of osteocalcin (OC), bone-specific alkaline phosphatase (BALP), and CTx were significantly correlated with a one year increase in BMD at the lumbar spine.[316] A positive correlation was found between OC and increased BMD at the femoral neck.[316] During the process of skeletal accretion, OC levels have been shown to reflect bone growth rates,[252] and maximal OC levels are reached in early puberty.[326] Bonofiglio *et al.* found that OC was inversely related to radial BMD.[327] BALP and OC are inversely correlated with BMD.[328] It is known that 1,25(OH)2D has effects on osteocalcin. Prior to menarche, girls' pubertal status and bone age were positively associated with IGF-I concentrations,[327] which may also be regulated by 1,25(OH)2D. A role for 1,25(OH2D is suggested as well as a possible explanation for increased 1,25(OH)2D concentrations during puberty.

In a recent study, concentrations of OC were highest at Tanner stages 2 and 3 for both sexes,[263] indicating an increased rate of formation at this pubertal stage. Fares *et al.* found that girls (13 ± 2 years) with insufficient 25(OH)D concentrations had significantly higher serum levels of BALP and CTx in comparison with girls who were vitamin D sufficient.[263] Analysis revealed no effect of vitamin D on bone remodeling. As noted by the authors, the increase in bone resorption markers associated with vitamin D insufficiency may still indicate a deleterious effect on bone remodeling. Failure to achieve statistical significance may have been be due to the small sample size of the subgroups. A positive correlation between 25(OH)D concentrations and CTx had been previously demonstrated by El-Hajj *et al.*[329]

These findings indicate that 1,25(OH)2D concentrations are increased during the peak growth spurt and that increased 1,25(OH)2D correlated with bone mass acquisition, presumably for calcium absorption and retention, though an increased demand of 25(OH)D may relate to increases in muscle mass as well.[234] It may be possible that 1,25(OH)2D is required for the growth of all tissues given its known involvement in the cell cycle,[330–333] however the extent of involvement is unclear. Nuclear actions of 1,25(OH)2D on muscle cells may be responsible for the positive association of 25(OH)D and 1,25(OH)2D with muscle strength and function in adults.[334–336] Similar roles for vitamin D may exist during growth of muscle and bone tissues. An increased demand for 1,25(OH)2D during puberty may be considered a consequence of rapid growth.

D. Vitamin D in Adolescents: Insufficiency and Supplementation

Family studies suggest that approximately 80% of the variability in peak bone mass can be explained by endogenous factors (genetics and hormones). Although exogenous factors (nutrition and lifestyle) play a less important role, unlike genetics, nutritional factors can be readily manipulated to improve mineral status. Vitamin D inadequacy during the pubertal growth spurt is speculated to result in a failure to achieve PBM, which likely contributes to the development of osteoporosis later in life. The rationale is that when vitamin D status is insufficient, levels are inadequate to maintain serum calcium concentrations, which results in hypocalcemia, which leads to secondary hyperparathyroidism. Hyperparathyroidism, in turn, leads to increased 1,25(OH)2D concentrations to normalize calcium. Increased 1,25(OH)2D and PTH, which stimulate bone resorption, are maintained at the expense of bone integrity.

For adolescents the recommended dietary intake of vitamin D is 5 μg (200 IU)/day. As shown in Table 6, insufficient intakes are quite common, with mean values well below recommended intakes. McKenna reported that the mean vitamin D intake is significantly lower in Central Europe (2.5 μg/day) when compared with North America (6.2 μg/day) or Scandinavia (5.2 μg/day), in which vitamin D intakes appear to be adequate.[337] Jones *et al.*, in Tasmania, Australia, have demonstrated that during summer, 8-year-old children had a mean 25(OH)D concentration of 79 nmol/L that did not correlate with dietary vitamin D intake, highlighting the significant contribution of sunlight as a major determinant of vitamin D stores in children.[27] Many studies demonstrate a seasonal variation of 25(OH)D in adolescents throughout the world (Table 6). The calculated non-weighted mean 25(OH)D level in 3,584 children and adolescents was 43 nmol/L in the winter and 70 nmol/L in 2,200 children in the summer.[34] Guillemant *et al.* clearly show 25(OH)D seasonal variation in a group of boys, ages 13–16 years, with winter values significantly lower than those found during the summer months.[319] In adults, Woitge *et al.* have pointed out seasonal variation in 25(OH)D concentrations, as well as an impact of this variation on bone formation and resorption markers, including: 1,25(OH)2D, PTH, pyridinoline (PYD), deoxypyridinole (DPD), and NTx, which were shown to vary by season.[338] Changes in bone markers are likely to be directly related to variation in 25(OH)D as a result of the demand for 1,25(OH)2D. At a time when skeletal growth is maximal, seasonal variation of vitamin D stores may limit the potential of reaching PBM, especially during winter months.

Vitamin D production is limited to the summer months at latitudes above 51°.[339] Vitamin D seasonal variation and winter-time insufficiency are not unexpected, but the evidence suggests that the prevalence of vitamin D insufficiency is much more common than it should be and may have crucial effects on bone development. Tylavsky *et al.* recently reviewed these data, but we have

found the incidence of vitamin D insufficiency in adolescent populations to be more common than was revealed by the aforementioned article.[340] Table 6 demonstrates that significant insufficiency occurs at latitudes as low as 33.5°N, with clear seasonal variations in 25(OH)D concentrations.[341] In adolescents, mean 25(OH)D levels at the end of winter were 42.5 nmol/L at 33.5°N,[329] 33.75 nmol/L at 43.5°N,[228] 20.5 nmol/L at 49°N,[319] and 33.9 nmol/L at 60°N.[317] This suggests that vitamin D production is limited even at latitudes that permit longer seasons of vitamin D photosynthesis. Rates of vitamin D insufficiency in adolescent populations were seen to be as high as 97% in Finland.[342] Ginty *et al.* found a greater incidence of vitamin D insufficiency occurred, in general, in older adolescents,[256] which supports the role of an increased requirement for vitamin D in skeletal growth.

In adults it is well established that vitamin D insufficiency leads to secondary hyperparathyroidism, bone loss, and higher risk for fractures,[343–345] while vitamin D and calcium repletion have been shown to decrease fracture rates.[346,347] During childhood and adolescence, reduced remodeling is also associated with decreases in BMD.[295] The skeleton serves as a nutrient reserve for calcium and phosphorus and high levels of remodeling, which could result from high serum PTH concentrations, may be deleterious to bone mass accretion. In adolescents with vitamin D insufficiency, PTH secretion increases to adapt to the higher rates of bone formation associated with growth. In the subgroup of Lebanese girls with the lowest levels of 25(OH)D (<50 nmol/L), serum levels of BALP and CTx were the highest, although these differences did not persist in the adjusted analyses.[263]

Jones *et al.* have demonstrated a positive correlation between bone mass and sun exposure in prepubertal children,[296] suggesting that replete vitamin D levels have an important role in skeletal health. BMD in Jewish adolescents (ages 15–19 years, at 40°N) was significantly lower in boys than in girls at the lumbar spine and was accompanied by poor vitamin D status.[348] The mean serum concentration of 25(OH)D was 46 ± 19 nmol/L.[348] Normal 1,25(OH)2D concentrations were found and may represent a compensatory mechanism allowing calcium absorption in the intestine at the cost of depleted 25(OH)D stores. A large percentage of female adolescents (ages 14–16 years) with low vitamin D status had low BMD values at both the radial and ulnar sites.[349] Similarly, El-Hajj Fuleihan *et al.* demonstrated a significant association between 25(OH)D concentrations and BMD of the spine, femoral neck, and radius.[341] Further, bone density has been found to be affected by the amount of winter solar exposure in Tasmanian children (8 years of age).[296] However, the extent to which seasonal deficiency of vitamin D affects skeletal mineralization and peak bone mass acquisition is uncertain. Not all observational studies have consistently shown a positive relationship between serum 25(OH)D levels and bone.[34,350] The convincing evidence for a positive impact of vitamin D on bone should be provided by randomized controlled trials (see below).

1. Effect of Vitamin D Supplementation on Serum 25(OH)D Levels

There are only a handful of trials evaluating the effect of vitamin D supplementation on serum 25(OH)D levels in adolescents. Docio *et al.* demonstrated that 40 μg (1,600 IU)/day of vitamin D3 for a period of one week was not enough to prevent vitamin D insufficiency in winter, and 80% of participants had 25(OH)D concentrations <50 nmol/L, with 31% <30 nmol/L.[228] African-American children (n = 5) were treated with 5 μg (200 IU)/day of vitamin D3 for one month an amount insufficient to raise 25(OH)D concentrations.[236] Lehtonen-Veromaa *et al.* supplemented 171 girls (ages 9–15 years) from October to January in Turku, Finland (60°N), with 10 μg (400 IU)/day vitamin D2 to no avail.[351] French boys (ages 13–16 years, n = 54) were supplemented with 2,500 μg (100,000 IU) vitamin D every two months during the winter (end of September, November, and January). At an intake of roughly 42 μg (1,670 IU)/day of vitamin D3, serum 25(OH)D concentrations were maintained at baseline summer values.[319] This suggests that adolescents at higher latitudes require almost 45 μg (1,800 IU)/day to avoid becoming vitamin D deficient during the winter.

2. Effect of Vitamin D Supplementation on Musculoskeletal Parameters

American girls (ages 9–13 years, n = 98) were randomized to receive low vitamin D and calcium dairy (3.2 μg (128 IU)/day and 728 mg/day, respectively) or high vitamin D and calcium dairy (7.2 μg (288 IU)/day and 1437 mg/day, respectively). Serum 25(OH)D did not differ significantly between groups receiving 3.2 μg (128 IU)/day and 7.2 μg (288 IU)/day at the end of the study; however, gains in BMD of the lumbar spine and total body correlated with calcium and vitamin D intakes, and vitamin D intake was also associated with bone mineralization.[352] The effect on bone of regularly consuming dairy products containing 560 mg calcium, with or without 5–8 μg (200–300 IU) of vitamin D, was compared with placebo in Chinese girls (ages 10–12 years, n = 698). Total-body BMD and BMC values were greater in the calcium with vitamin D group than in either the calcium alone or placebo grops.[353] Two recent randomized trials evaluated the impact of vitamin D supplementation alone on bone mass in adolescents. In Finnish girls (ages 11–12 years, n = 228), no differences were noted at one year in changes in bone mass at the spine or femur in the intent-to-treat analyses, but a positive effect could be demonstrated in the per-protocol analyses using doses of 5 or 10 μg (200 or 400 IU)/day.[354] In the second study, 169 Lebanese girls (10–17 years of age) were randomized for one year to placebo (n = 55), 5 μg (200 IU)/day of vitamin D3 (n = 58), or 50 μg (2,000 IU)/day of vitamin D3 (n = 55).[341] Larger increments in bone mass, bone area, and lean mass were noted in both vitamin D3 groups than in the placebo group.[341] Multivariate analyses revealed that the positive impact of vitamin D supplementation on bone mass in these girls was in large part mediated by vitamin D-induced

changes in lean mass and bone area.[341] A trend was noted for the largest increments in bone mass acquisition in girls with the lowest vitamin D levels at study entry, in prepubertal girls, and in the subgroup taking the higher dose of vitamin D3.[341]

E. Determining an Optimal Concentration of 25(OH)D for Adolescents

There is no consensus on the definition of optimal vitamin D status in adolescence or adulthood. In fact, great variation exists between reports when defining "deficient," "insufficient," or "adequate" levels of vitamin D. Rickets is associated with 25(OH)D concentrations <25 nmol/L, for which the most cases of radiologically proven rickets are found.[355] Definitions of insufficiency based on different criteria range from 25 nmol/L to 75 nmol/L, with sufficient levels being considered >37.5 to >75 nmol/L [356] (various definitions are demonstrated in Table 6). The lack of consistency and the lack of a good biomarker to define optimal vitamin D status make distinguishing the reports a challenge.

As previously discussed, the classical function of vitamin D is to enhance calcium absorption. In the context of puberty, an extremely important time of life for bone growth, the importance of understanding the dietary requirements of vitamin D becomes clear. There are several methods used in adult trials to attempt to determine a circulating 25(OH)D concentration that is adequate; these include maximal suppression of PTH levels, greatest calcium absorption, highest BMD, and reduced rates of bone loss, falling, and fractures. A recent review summarized the available data for each method in an adult populations (6). Similarly, we have applied these methods to adolescent trials in an attempt to determine an optimal 25(OH)D concentration for bone health for this age group.

Serum 25(OH)D and PTH are inversely correlated in a dose-response manner. Suppression of PTH, due to its potentially harmful effects on bone, has been utilized in several adult studies. As demonstrated by Dawson-Hughes *et al.,* recommended 25(OH)D concentrations in adults in the 75–80 nmol/L range have been found to be most prevalent in suppressing PTH.[6] There is an intuitive concern that low PTH concentrations may inhibit bone growth, and thus increasing 25(OH)D concentrations above a threshold may be detrimental to bone health. There is no evidence to support this. The more likely scenario is that increasing 25(OH)D concentrations improves the body's response to PTH, such that less PTH is required for the same response. Increased 25(OH)D does not change calcium homeostasis to a detectable degree, but PTH response is altered. Suppression of PTH levels to the low end of normal, still within a physiological range, is therefore appropriate.[357]

In adolescents, 25(OH)D concentrations >50 nmol/L have been demonstrated to stabilize PTH levels.[228] Guillemant *et al.* clearly show that

winter-time vitamin D insufficiency is associated with an increase in PTH, and they further demonstrate that 100,000 IU of vitamin D3 given every two months during winter (approximately 1,667 IU/day) is sufficient to prevent the dramatic winter-time increase in PTH concentrations seen in placebos.[358] An increase in PTH levels still occurred at serum 25(OH)D concentrations between 50 and 60 nmol/L, suggesting that 25(OH)D concentrations required to maximally suppress PTH in adolescents are >60 nmol/L.[358] Similar results were found in a population of 6–10-year old African-American children, in whom it was noted that stable PTH levels were achieved when 25(OH)D levels exceeded 75 nmol/L.[236] In a cross-sectional analysis of Finnish adolescent girls (ages 14–16 years, n = 178), Outila *et al.* failed to reveal a plateau in PTH levels associated with 25(OH)D concentrations <40 nmol/L. However, when 25(OH)D concentrations were >40 nmol/L, a mean serum PTH concentration of ~30ng/L was reached in a population in which 5% of subjects had PTH concentrations >55 ng/L.[349] Forty-six per cent of Finnish girls (ages 10–12 years, n = 193) in which vitamin D insufficient (<40 nmol/L), with significantly higher PTH concentrations consistent with secondary hyperparathyroidism, indicating that concentrations of 25(OH)D in excess of 40 nmol/L are required.[230] El-Hajj Fuleihan *et al.* noted in adolescents that when 25(OH)D concentrations were 50–75 nmol/L, very few PTH levels were found above the normal range.[359] For maximal PTH suppression in adolescents, like adults, 25(OH)D concentrations exceeding 75 nmol/L are required.

Increased calcium absorption mediated by improved vitamin D status has been used as an indicator for optimal vitamin D levels. Adults with mean 25(OH)D concentrations of 86.5 nmol/L had 65% greater calcium absorption than those with 25(OH)D <50 nmol/L.[360] In adolescents, Abrams *et al.* demonstrated an interaction of serum 25(OH)D with PTH in determining the calcium absorption fraction.[361] 25(OH)D concentrations <62.5 nmol/L showed a significant negative relationship between 25(OH)D and fractional calcium absorption, suggesting that levels >62.5 nmol/L are required for optimal calcium absorption in adolescents.

A relationship between 25(OH)D concentrations and bone density has been determined. In a cohort of 13,432 men and women, serum 25(OH)D concentrations and total hip BMD were positively associated up to 25(OH)D levels of 90–100 nmol/L.[362] Bone loss may also be preventable with adequate vitamin D and calcium. Wintertime bone loss from hip and spine in adults was found to be reduced when 25(OH)D levels were 60–90 nmol/L.[363,364] From these findings, 25(OH)D levels required to optimize bone density in adults may be 60–100 nmol/L.

There is poor understanding of the relationship between 25(OH)D and optimal skeletal mineralization during adolescence. Vitamin D-deficient Finnish girls (12–14 years of age, n = 193) were found to have lower cortical bone density, higher PTH concentrations, and increased concentrations of

tartrate resistant alkaline phosphatase 5b, a bone resorption marker.[230] These findings indicate that 25(OH)D concentrations <25 nmol/L may limit the accrual of bone mass. Lower mean radial BMDs were found when 25(OH)D concentrations were ≤40 nmol/L when compared with >40 nmol/L. In addition, a positive relationship has been demonstrated between serum 25(OH)D concentrations and lumbar spine and femoral neck BMD in adolescents.[233] Serum 25(OH)D concentrations >25 nmol/L are required to prevent bone resorption, and concentrations of at least >40 nmol/L are required for a positive effect on BMD in adolescents.

The risk in elderly adults of fracturing a bone has been shown to be associated with vitamin D status. Increased 25(OH)D levels have been associated with a reduction in the number of falls in older individuals. A meta-analysis found a 26% reduction in risk of hip fracture with supplementation of 17.5–20 μg (700–800 IU)/day vitamin D.[365] In addition to an effect on fractures, adequate vitamin D may have further benefits by preventing falls that cause fractures in elderly people. Supplementation with 20 μg (800 IU)/day vitamin D3 was shown to reduce the risk of falling by 22%.[366] The effect of vitamin D on the risk of falling is attributed to its effects on musculoskeletal function.

Fracture data in adolescents, though available, have not been associated with 25(OH)D concentrations to our knowledge. Inadequate 25(OH)D concentrations result in limited levels of substrate for conversion to 1,25(OH)2D. During childhood, and especially in adolescence, increased concentrations of 1,25(OH)2D occur during periods of skeletal growth. Supplementation with 25(OH)D produced increases in 1,25(OH)2D concentrations when basal levels of 25(OH)D were insufficient; the reflection point was found to be around 50 nmol/L for 25(OH)D,[228] suggesting that using the above criterion, 50 nmol/L is the minimal desirable concentration of 25(OH)D.

In conclusion, common opinion has suggested that an optimal serum 25(OH)D concentration of 70–80 nmol/L is required for bone health in adults (6). A report that compares the serum 25(OH)D versus PTH relationship for data sets from various age groups shows no substantial difference between children and adults.[357] To attain a mean level of 75 nmol/L requires a daily intake of 20–25 μg (800–1,000 IU) vitamin D3 (6). In adolescents, serum 25(OH)D concentrations in the range of 40–75 nmol/L are required to keep PTH levels within normal ranges: >40 nmol/L to begin a positive association with BMD; and >62.5 nmol/L to maximize calcium absorption, and levels close to 100 nmol/L to optimize bone mass accrual.[341] This suggests that 25(OH)D concentrations of >75 nmol/L may be required for optimal bone health in adolescence. In children, similarly to adults, the mean 25(OH)D response to each μg (40 IU) of additional oral vitamin D3 is approximately 1 nmol/L.[34,341,354] This would require a daily intake of vitamin D3 around 30–40 μg (1,200–1,600 IU)/day; the range depending on lifestyle

and gender of the subject. More research in this area would greatly enhance the basis for such a recommendation.

F. Consequences of Vitamin D Deficiency and Insufficiency

1. Rickets

Rickets was epidemic during industrialization in the early eighthteenth century in northern Europe from a general lack of sunlight. The prevalence of rickets during the late eighthteenth and nineteenth centuries was 40–60% in inner-city children of northern Europe.[367] After the discovery of treatment for vitamin D-deficiency rickets with sunlight, the therapeutic benefit of cod liver oil, the isolation of vitamin D, and fortified dairy products, the incidence of rickets was greatly diminished. Today rickets is again becoming a public health problem despite effective, inexpensive methods of prevention. Over the past 20–30 years, there has been a reemergence of rickets with reports in the United Kingdom (UK),[368–370] Europe,[371–373] and North America[374] in a variety of ethnic groups. Rickets continues to be endemic in Africa, where the disease is more strongly attributed to a lack of calcium.[375,376] From 2002–2004, the mean number of rickets cases reported in Canada was 14 ± 2.1 per month between February 1 and May 31 and 6.9 ± 2.1 cases per month between June 1 and January. 31.[377] The overall incidence of vitamin D-deficiency rickets was 2.9 per 100,000 children, with the majority being breast-fed (94%), non-Caucasian infants.[377] Thacher *et al.* state that "nutritional rickets has been described from at least 59 countries in the last 20 years".[378] Not surprisingly, the resurgence of rickets in North America in the 1990s coincided with skin cancer prevention campaigns.[379] Other possible reasons for the increasing prevalence of rickets include: breastfeeding without vitamin D supplementation, vegetarian diets,[380] darker-skinned people migrating to countries with less sunlight,[372,381,382] and increasing atmospheric pollution.[383]

Rickets develops most commonly during the early months of life. The early signs consist of growth failure, lethargy, and irritability, followed by more detectable clinical changes such as costochondral beading, swelling of the distal ends of long bones (wrists and ankles), and bowing of the legs.[384] Hypocalcemia can cause convulsions, stridor and neuromuscular irritability (spasms),[385] and fractures may occur.[386] In children 1–3 years of age, stunting of growth, bowing of the legs, muscle weakness, walking problems, and deformation of the pelvis signal vitamin D deficiency. Healing of skeletal deformities can be reversed by vitamin D treatment.[387]

In adolescence, rickets can occur during the pubertal growth spurt.[388] A waddling gait, lower limb and back pain, bowing of the legs, and muscle weakness are common symptoms.[384] Hypocalcemic tetany is also common.[388] Skeletal deformation is often permanent after late rickets occurs despite corrective treatment with vitamin D, emphasizing the need to ensure adequate

vitamin D intake during adolescence. Heaney point out that rickets reflects severe vitamin D deficiency and that milder degrees of insufficiency may not produce clinical symptoms but cause reduced efficiency in utilization of dietary calcium, impeding full acquisition of the genetic potential for bone mass.[389]

There is a high prevalence of rickets in the Asian community of the UK. The predisposition of this community to rickets and osteomalacia [390] has been attributed to several combined pathogenic mechanisms, including lack of sunlight exposure, increased skin pigmentation, lack of dietary vitamin D intake, genetic predisposition, low-calcium diets, and high dietary phytate content. Clements *et al.* proposed that the pathogenesis of rickets in the UK Asian community is attributable to the high-cereal, low-calcium diet, which induces mild hyperparathyroidism and elevation of 1,25(OH)2D concentrations, with a resultant reduction in vitamin D status.[391] Evidence in rats shows that low-calcium or high-phytate diets resulted in increased catabolism of 25(OH)D to inactive metabolites and increased excretion of these products in the stool, all of which resulted in a reduction of 25(OH)D stores.[392] The primary cause of rickets can be a severe deficiency in either vitamin D or calcium or a combination of moderate deficiency in both simultaneously.[375,393] Figure 4 illustrates the key nutrient relationships that affect the risk for and treatment of rickets. According to Lerch and Meissner rickets can be prevented with either vitamin D or with calcium, and either approach will move the risk out of the rickets zone illustrated in Figure 4.[393] Reduced sun exposure, increased skin pigmentation, and/or limited dietary vitamin D or calcium intake combined with the reduction in 25(OH)D half-life, is sufficient to produce vitamin D deficiency and rickets.

Bonet *et al.* reported on three Pakistani teenage immigrants in Barcelona, Spain, who presented with limb pains, muscular weakness, knock-knee, and

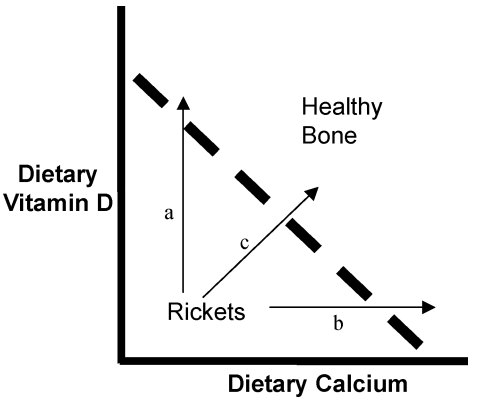

FIGURE 4 Schematic representation of the nutrient relationships that affect the risk of rickets. Nutrient-deficiency rickets is the result of a lack of vitamin D and/or calcium. The light arrows represent the pathways by which rickets can be treated, with either (a) vitamin D, or (b) calcium, or (c) with both.

seizures; all were subsequently diagnosed with rickets.[371] Vitamin D treatment and dietary counseling normalized biochemical measurements and improved symptoms. Narchi *et al.* have described 21 adolescent girls diagnosed with symptomatic nutritional rickets.[388] Hypocalcemia, hypophosphatemia, elevated serum alkaline phosphatase, and serum PTH with reduced 25(OH)D levels were found and compared with 20 normal controls who had >60 min/day of sun exposure. Vitamin D deficiency was ascribed to significantly less sun exposure in girls with rickets.[388] Reports such as these should signal a need for increased vigilance and awareness of vitamin D nutrition.

2. Chronic Disease

According to Osteoporosis Canada (www.osteoporosis.ca), 1.4 million Canadians (about 5% of the population) suffer from osteoporosis, with a staggering $1.3 billion (Cdn) spent on treatment per year. The causes of osteoporosis are multifactorial and include poor PBM, gender, genetics, and nutrient intake.[394] A reduced bone mass in osteoporosis is the foundation of the strategy to maximize PBM during adolescence in an attempt to prevent osteoporosis in adulthood. During childhood and adolescence the accumulation of lifetime maximum bone helps to prevent bone mass from declining later in life to the critical level that defines osteoporosis (-2.5 standard deviations below the PBM of young women).[283]

As previously discussed, the positive effect of vitamin D on bone mass and fracture prevention is well established in adults.[365] Mounting evidence from observational studies in neonates, children, and adolescents, as well as from randomized trials in adolescents, underscores the importance of vitamin D to musculoskeletal health during youth. Such evidence supports the institution of targeted primary intervention strategies from childhood to adolescence—critical periods of skeletal maturation—as effective measures to maximize bone and thereby to minimize the risk of osteoporotic fractures later in life.

Although evidence for these skeletal effects is preliminary, there are many other potential benefits to adequate vitamin D status. Vitamin D may affect risk for other diseases, both acute and chronic. Vitamin D insufficiency has been linked with the risk of influenza, and tuberculosis[395,396] as well as autoimmune diseases and a variety of cancers.[82,397–413]

Geographic distributions of diseases, such as multiple sclerosis (MS), prostate cancer, colon cancer, breast cancer, and type 1 diabetes mellitus (DM) overlap with regions characterized by limited vitamin D production (latitude and sunlight hours). It is the non-calcemic actions of vitamin D that are hypothesized to play a role in chronic disease development and progression. It has been suggested that higher 25(OH)D concentrations are required for optimal extraskeletal effects. One reasonable model for

vitamin D metabolism depicts "normal" circulating 25(OH)D levels as suboptimal for extrarenal conversion to 1,25(OH)D, thereby leaving non-classical cells/tissues deficient, which may influence the development of disease (Figure 5).[43] Currently defined "normal" 25(OH)D concentrations are directed solely toward maintaining calcium homeostasis; more specifically, these levels are required to prevent disease caused by severe vitamin D

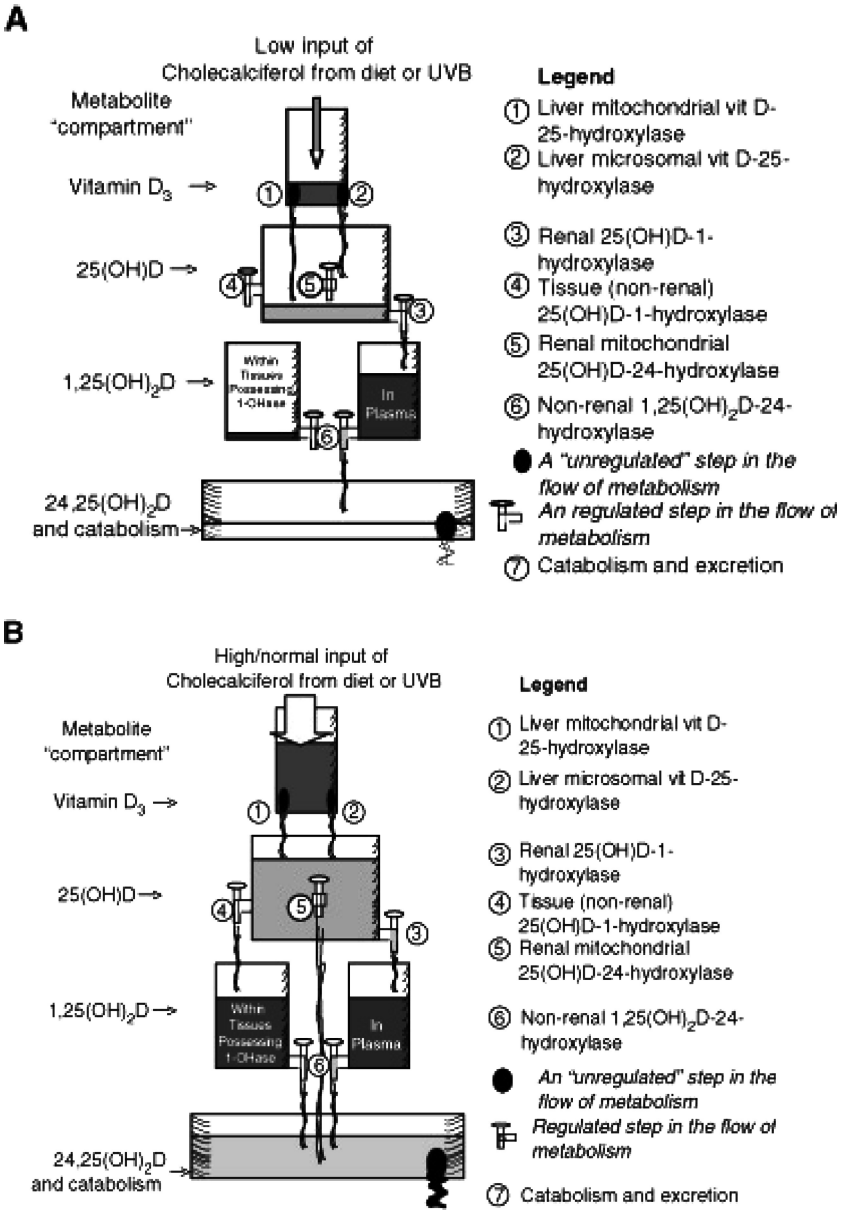

FIGURE 5 Consequences of low (A) and adequate (B) 25(OH)D concentrations (from Ref. 43, used with permission).

deficiency. Thus, vitamin D-deficient diseases related to bone health (rickets, osteomalacia, and osteoporosis) may be avoided, but "deficiency" may still be present.

Mounting evidence implicates insufficient vitamin D status as a risk factor for autoimmune diseases, including rheumatoid arthritis (RA), MS, systemic lupus erythematosis (SLE), and type 1 DM. Vitamin D intake has been inversely associated with RA,[414] and a protective effect of vitamin D intake on the risk of developing MS has been demonstrated.[415] Low circulating 25(OH)D levels have been revealed in both SLE[416–418] and MS.[419–422] A Finnish study demonstrated an association between vitamin D supplementation in infancy with a reduced risk for type I DM (208); moreover, low circulating levels of 25(OH)D have been demonstrated to be associated with the risk of both type I[423] and type 2 DM.[424,425] In patients with MS, seasonal fluctuations in disease activity have been demonstrated by magnetic resonance imaging, and these fluctuations have been related to seasonal UVB production of vitamin D.[426] Further, allelic variations in the *VDR* gene may also contribute to these disease states. *VDR* polymorphisms have been associated with MS[427–429] and type 1 DM[430] susceptibility. The prevailing hypothesis for vitamin D's relation to autoimmunity is that low circulating 25(OH)D concentrations, though able to maintain calcium homeostasis, are restrictive to local paracrine conversion to, and use of, 1,25(OH)2D in tissues such as the brain and lymph nodes, which may contribute to chronic disease by impairing proper regulation/development/proliferation of the respective system.

It has been demonstrated that 1,25(OH)2D can positively regulate expression of certain genes by binding VDREs present in their promoter regions[431,432] and negatively regulate expression[433] by antagonizing the action of certain transcription factors, such as NF-AT and NF-κB.[434,435] Immunoregulation by 1,25(OH)2D is accomplished by inhibition of cytokine expression. 1,25(OH)2D inhibits expression of IL-2 (T lymphocytes and peripheral blood mononuclear cells (PBMCs)),[436,437] TNF-α (PBMCs), and IFN-γ (PBMCs).[438] *In vitro*, 1,25(OH)2D modifies T-cell differentiation, and dendritic cell activation, and it induces a shift in the profile of cytokines secreted during an immune response to an anti-inflammatory phenotype, with regulatory T lymphocytes predominating in the cell population.[439]

In vitro, 1,25(OH)2D renders dendritic cells more tolerogenic by inhibiting antigen presentation and down-regulating co-stimulatory molecules, as well as down-regulating IL-12 production and secretion.[413,440–443] These effects, in turn, result in the inhibition of destructive T lymphocyte proliferation characteristic of inflammatory disease. PBMCs from SLE patients treated *in vitro* with 1,25(OH)2D have shown significantly reduced proliferation, reduced immunoglobulin production, and induced apoptosis of activated B cells.[444] These effects of 1,25(OH)2D may be beneficial in autoimmune disease.

In animal models of MS and type 1 DM, experimental allergic encephalomyelitis (EAE), and non-obese diabetic (NOD) mice, 1,25(OH)2D has been shown to prevent the development of disease. The dominant features in MS include autoreactive T lymphocytes, axonal injury, and degeneration in the brain. In EAE mice, 1,25(OH)2D supplementation can prevent EAE and attenuate symptoms when active disease is present,[445,446] probably by limiting the occurrence of activated monocytes [447] and autoreactive T lymphocytes[448] in the central nervous system. It has also been speculated that EAE is prevented with 1,25(OH)2D treatment by stimulating apoptosis of inflammatory cells.[449] 1,25(OH)2D also prevents the development of diabetes in NOD mice (443,450–452). Riachy *et al.* have recently demonstrated that 1,25(OH)2D down-regulated the Fas death receptor, thereby protecting the beta-cells of the pancreas (responsible for insulin secretion) from cytokine-induced apoptosis, the dominant feature of type 1 DM.[453] Cholecalciferol supplementation in spontaneously hypertensive rats and Wistar rats has been shown to reduce blood glucose levels,[454] further indicating that supplementation with vitamin D may be effective in the prevention of insulin-resistance associated with type 2 DM.

In humans, Goldberg *et al.* demonstrated a beneficial effect when MS patients were supplemented with 125 μg (5,000 IU)/day of vitamin D3 (in the form of cod liver oil); patients had a significant decrease in the number of relapses experienced.[455] Wingerchuck *et al.* similarly demonstrated a benefit, reducing the relapse rate in 15 MS patients by 27%, when patients were treated with 2.5 μg/day of 1,25(OH)2D for 48 weeks.[456] Though these results indicate a therapeutic role for vitamin D in MS, dose-finding studies have not yet been completed. Recently, Pittas *et al.* also demonstrated a beneficial effect of calcium and vitamin D supplementation in decreasing indices of insulin resistance in high-risk subjects in an osteoporosis trial.[424,457] Adults whose infant medical records indicated they had received the Finnish recommended daily allowance for vitamin D in the 1960s (2,000 IU/day) were one-sixth as likely to be type 1 juvenile diabetics by age 30 years.[208]

If theory can be translated into results, supplementation with vitamin D may be beneficial in some or all of these autoimmune disorders. The optimal dose required for the desired effect must be investigated before randomized controlled trials are undertaken. The therapeutic potential of vitamin D should be investigated the same as that of any drug. Appropriate clinical trials should be undertaken to determine conclusively the merits of vitamin D's use as a disease-modifying agent. Questions pertaining to drug logistics must arise. Supplemental doses in the above trials were chosen purely arbitrarily. Dose recommendations must also consider age-related variations, the particular disease, and therapeutic versus prevention studies.

VI. CONCLUSION

Rickets is the most overt sign of vitamin D deficiency, yet despite long-standing preventive recommendations, its incidence persists at 2.9 per 100,000 children in Canada. There are new concerns that less severe but long-term inadequacy of vitamin D early in life may have life-long consequences relating to increased risk of chronic disease. Adequate vitamin D status during pregnancy is important for the long-term bone health of the offspring. Clinical trial evidence relating to the effects of vitamin D supplementation during pregnancy is limited because of ethical difficulties in studying this population. Cross-sectional findings based on serum 25(OH)D concentrations suggest that vitamin D intakes up to 50 μg (2,000 IU)/day may be desirable for proper vitamin D nutrition during pregnancy.[76,458] In northern communities, vitamin D insufficiency (<50 nmol/L) was found in 76% of mothers and 43% of their infants.[176] Increased awareness about vitamin D nutrition is necessary.

The data pertaining to vitamin D nutrition during prepubertal years are scarce. Concentrations of 1,25(OH)2D increase with age. There is some evidence to indicate that serum 25(OH)D levels decease with age in relation to increases in fat-free mass, suggesting the vitamin D nutrition is involved in muscle accumulation as well as bone growth. The available clinical trials demonstrate that 10 μg (400 IU)/day is not enough to maintain serum 25(OH)D concentrations in this age group.[236,237]

Adolescence is an important time for skeletal growth and muscle accumulation. The greatest increases in bone mass have been shown to occur in tandem with the highest concentrations of 1,25(OH)2D, likely reflecting the need to absorb calcium from the gut. Winter supplementation of adolescents with 10 μg (400 IU)/day failed to maintain serum 25(OH)D concentrations.[228,351] In French boys, approximately 42 μg (1,670 IU)/day was required to maintain winter 25(OH)D concentrations. The gain in bone density in girls has been shown to be increased with higher vitamin D supplementation.

We applied the criteria established by Dawson-Hughes *et al.* to define an optimal 25(OH)D concentration. Similar to adults, based on clinical trial and cross-sectional data, there is moderate evidence to suggest that serum 25(OH)D concentrations above 75 nmol/L are required for optimal skeletal health in adolescents. Intakes in the range of 30–40 μg/day (1,200–1,600 IU/day) may be required to reach these concentrations. However, mean 25(OH)D concentrations in adolescent populations usually fall well below this level. An increased awareness is required pertaining to the need for vitamin D supplementation, physicians should be screening serum 25(OH)D concentrations, especially in children with dark skin. Further research is needed to conclusively determine optimal 25(OH)D concentrations during pregnancy through to adolescence and the required doses to attain these concentrations.

Osteoporosis is a common health problem that usually manifests in later decades of life with a significant impact on health-care budgets. One likely means of reducing the risk of osteoporosis is to optimize nutritional intake of calcium and vitamin D during adolescence to maximize peak bone mass. In addition to bone disorders, early life vitamin D status may influence development and reduce the risk for chronic diseases, such as multiple sclerosis and juvenile diabetes. The positive health effects of an adequate vitamin D status are only beginning to be uncovered.

In conclusion, poor vitamin D nutrition should be a universal concern. There is an urgent need for randomized trials to determine an optimal vitamin D status from fetal life through to adolescence. Furthermore, public health awareness and a clear policy for clinicians and health-care workers are needed concerning screening and maintaining sufficient vitamin D status.

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