Vitamin D in health and disease: a literature review

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ABSTRACT

Vitamin D, a fat-soluble prohormone, is synthesised in response to sunlight. Vitamin D requires two metabolic conversions, 25-hydroxylation in the liver and 1α-hydroxylation in the kidney, to become active hormone. The active form, 1α,25-$(OH)_2$D, binds to the vitamin D receptor (VDR) to modulate gene transcription and regulate mineral ion homeostasis. Vitamin D plays several roles in the body, influencing bone health as well as serum calcium and phosphate levels. Furthermore, vitamin D may modify immune function, cell proliferation, differentiation and apoptosis. Vitamin D deficiency has been associated with numerous health outcomes, including risk of rickets in children or osteomalacia in adults, increased risk of fractures, falls, cancer, autoimmune disease, infectious disease, type 1 and type 2 diabetes, hypertension and heart disease, and other diseases such as multiple sclerosis. Here, vitamin D physiology and metabolism, its genomic action and association of polymorphisms in vitamin D pathway genes with different diseases are reviewed by focusing on new findings published in the literature.

KEY WORDS: Diabetes mellitus.
Genetics.
Neoplasms.
Polymorphisms.
Vitamin D.

Introduction

Vitamin D is a lipid-soluble prohormone that is vital for the maintenance of bone and muscle health by promoting the absorption and metabolism of calcium and phosphate.[1] In addition to food sources such as fatty fish, eggs, fortified milk and cod liver oil, the human body uses ultraviolet B (UVB) radiation from sunlight to synthesise a significant portion of vitamin D requirements.[2] There are two forms of vitamin D: vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol). The skin synthesises vitamin D_3 after sun exposure and it may be obtained from animal sources, while vitamin D_2 is the synthetic form that is often found in fortified food and is derived from plants.

The primary role of vitamin D has been considered to be the absorption of calcium from the intestine (i.e., calcium homeostasis in the body) and is necessary for skeletal health (bone mineralization, remodelling, and maintenance; Fig. 1). Over the years, it has become increasingly clear that vitamin D not only has a function in bones, but it also significantly affects cell proliferation and differentiation.[3]

Vitamin D is a global regulator of gene expression and signal transduction in virtually every tissue. In epithelial cells vitamin D, by binding with the vitamin D receptor (VDR), contributes to maintenance of the quiescent, differentiated phenotype and promotes pathways that defend cells against endogenous and exogenous stresses.[4]

Vitamin D physiology and metabolism

The synthesis of vitamin D starts with the oxidation of cholesterol to 7-dehydrocholesterol (7-DHC). 7-DHC is then transported to the skin and is stored in the cell membranes of keratinocytes and fibroblasts in the epidermis of skin. In the skin, 7-DHC is photolysed by UVB (280–320 nm) to previtamin D, which is converted to vitamin D by photolysis-mediated thermo-isomerisation.

To become biologically active, the vitamin D originating from dermal production or dietary sources undergoes a series of enzymatic conversions in the liver and kidney. Vitamin D is transported to the liver by vitamin D binding protein (DBP), which has a high homology to albumin, and vitamin D metabolites are mostly transported by DBP (85–88 %) and in part by albumin (12–15%).[5–7]

Vitamin D, bound to the vitamin D binding protein, is transported to the liver where the cytochrome P450 enzyme 25-hydroxylase (CYP2R1) adds a hydroxylgroup on carbon 25 to produce a major circulating form of vitamin D, (i.e., 25-hydroxyvitamin D [25-{OH}D] [Calcidiol]).[8–11] CYP2R1 is able to hydroxylate vitamin D_2 and vitamin D_3 at position 25.[10,12] Mutation in the *CYP2R1* gene results in rickets.[13] Strushkevich and colleagues have successfully crystallised the CYP2R1 enzyme with its vitamin D substrate bound in the active site.[12]

The inactive 25-(OH)D metabolite also circulates in the bloodstream bound to DBP and it must be further hydroxylated at a different site in the kidney tubules to gain hormonal bioactivity. Hydroxylation at position 1α by the mitochondrial cytochrome P450 enzyme 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) of kidney converts 25-(OH)D to 1α,25-dihydroxyvitamin D (1α,25-$(OH)_2$D; calcitriol), the most active hormonal form of vitamin D that plays an essential role in mineral homeostasis and is responsible for most of the biological action of vitamin D.[11,14–20]

The kidney is exclusively involved in synthesis of

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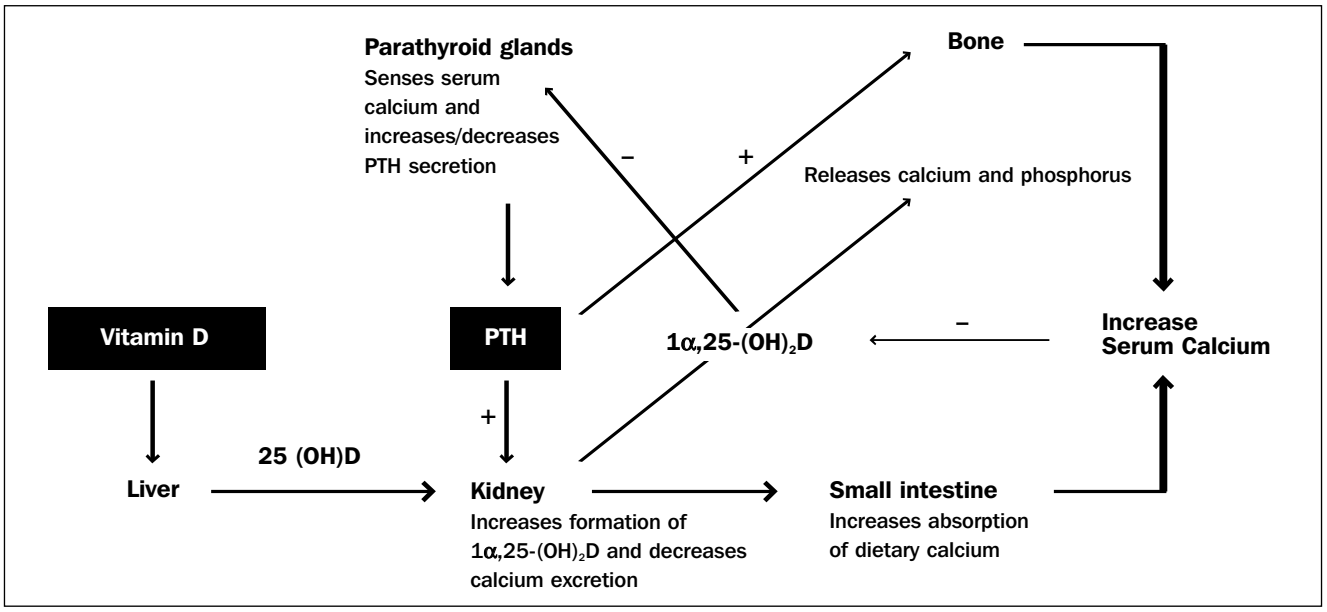

Fig. 1. Physiological role of PTH in the maintenance of serum calcium level. Key target organs for PTH – bone, kidney and intestine – and their feedback interactions with calcium are shown.

circulating 1α,25-$(OH)_2$D, as patients with chronic kidney disease exhibit low renal CYP27B1 enzyme activity and as a consequence have very low level of serum 1α,25-$(OH)_2$D. Kidney CYP27B1 is upregulated by parathyroid hormone (PTH) as part of calcium homeostasis and down-regulated by fibroblast growth factor (FGF23) as part of phosphate homeostasis. Mutation in the *CYP27B1* gene results in vitamin D-dependent rickets type 1 in humans and mice (Fig. 2).[21,22]

The renal enzyme cytochrome P450, 24-α-hydroxylase(CYP24A1), finally hydroxylates both 25(OH)D and1α,25$(OH)_2$D to initiate degradation of these vitamin D metabolites.[23–25] CYP24A1 is a multicatalytic enzyme responsible for a five-step, vitamin D inducible C-24-oxidation pathway which inactivates 1α,25(OH)2D to a water-soluble biliary form, calcitroic acid.[26,27] CYP24A1 also converts 25(OH)D into an inactive product, 24,25(OH)2D. Mutations in the *CYP24A1* gene have been associated with idiopathic infantile hypercalcemia (IIH).[28]

Mechanism of genomic action of vitamin D

The active form of vitamin D, 1α,25-$(OH)_2$D, has calcaemic and non-calcaemic roles. The calcaemic roles includes the regulation of blood calcium and phosphate concentrations by action in the intestine, bone, parathyroid and kidney, while non-calcaemic functions include cellular differentiation and antiproliferative actions in bone marrow (osteoclast precursor and lymphocytes), the immune system, skin, breast and prostate epithelial cells, muscle and intestine.[29] 1α,25-$(OH)_2$D exerts its biological effects in various cells both via non-genomic and genomic mechanisms. The genomic action starts with the interaction of 1α,25-$(OH)_2$D with VDRs, followed by subsequent interaction of VDR with other transcription factors such as coactivator proteins and transcription integrators such as calcium-binding proteins.[30] This genomic pathway leads to changes in gene transcription.[31] A more rapid response is achieved through interaction of vitamin D with a cell surface receptor and second messengers (Fig. 3).[29,31]

In brief, the above mentioned functions are performed by 1α,25-$(OH)_2$D through VDR-mediated transcriptional mechanism. Upon reaching a target tissue, 1α,25-$(OH)_2$D binds VDR that acts to regulate the transcription of vitamin D target genes responsible for carrying out the physiological actions of 1α,25-$(OH)_2$D.[32,33] VDR binds to DNA as VDR/VDR homodimers or VDR/RXR heterodimers in order to regulate gene expression.[34] The dimers subsequently recognise and bind with transcription factor IIB (TFIIB) to a vitamin D response element (VDRE) located in the promoter region of target genes and leads to transcriptional suppression or activation of vitamin D response genes.[35,36] VDR bound with 1α,25-$(OH)_2$D recruits a partner known as the retinoid X receptor (RXR) and a number of other transactivators to expose DNA and efficiently transcribe target genes.[37]

It has been estimated that 300–800 genes are regulated by 1α,25-$(OH)_2$D.[38] Among several target genes, the 1α,25-$(OH)_2$D hormone induces in target cells the expression of the gene encoding the key effector of its catabolic breakdown, 25-hydroxyvitamin D-24-hydroxylase (CYP24A1).[26,39] This ensures attenuation of the 1α,25-$(OH)_2$D biological signal inside target cells and helps regulate vitamin D homeostasis.

The detailed mechanism behind these physiological effects of vitamin D is described below. The genomic effects of vitamin D are mediated via binding to VDR, which is the only nuclear protein that binds the vitamin D_3 with high affinity.[40] The VDR contains two zinc finger motifs that collectively form a characteristic DNA-binding domain of 66 amino acids.[41] Additionally, the carboxy-terminal of the VDR protein contains a ligand-binding domain (LBD) of 300 amino acids formed by 12 α-helices.[42] The LBD is also involved in various interactions with nuclear proteins, such as other members of the nuclear receptor superfamily, CoA and co-repressor proteins.[43]

Co-repressor proteins (e.g., NCoR, SMRT and Alien) link non-ligand DNA-bound VDR to enzymes with histone deacetylase activity that causes chromatin condensation.[44]

Table 1. Serum 25(OH)D concentration and its interpretation.

Classification	Serum 25(OH)D	Clinical implications
Vitamin D deficiency	<50 nmol/L (<25 ng/mL)	Summarises concentrations in severe deficiency and insufficiency
Severe vitamin D deficiency	<25 nmol/L (<10 ng/mL)	Increased risk of rickets, osteomalacia, secondary hyperparathyroidism, myopathy, falls, fractures
Vitamin D insufficiency	<25–49 nmol/L (<10-19 ng/mL)	Increased risk of bone loss, secondary hyperparathyroidism
Adequate vitamin D threshold concentration	50 nmol/L (20 ng/mL)	Low risk for bone loss and secondary hyperparathyroidism, neutral effect on falls and fractures
Desirable vitamin D threshold concentration for fall and fracture reduction	75 nmol/L (30 ng/mL)	Optimal suppression of parathyroid hormone and bone loss, reduction of falls and fractures by about 20%

Adapted from Ofenloch-Haehnle, 2012.

This provides VDR with intrinsic repressive properties comparable to retinoic acid and thyroid hormone receptors. Binding of 1α,25-$(OH)_2$D with the LBD of VDR induces conformational change resulting in the replacement of co-repressor molecules by a CoA protein in complex with more general CoAs, such as CREB binding protein (CBP).[45,46] These CoA complexes have histone acetyltransferase activity which finally causes chromatin relaxation.[47]

In a subsequent step, ligand-activated VDR interacts with the mediator complexes, such as thyroid hormone receptor-associated protein 220 (TRAP220).[48] The mediator complexes, which consist of 15–20 proteins, build a bridge to the basal transcription machinery.[49] In this way, ligand-activated VDR performs two tasks, the chromatin modification and the transcription regulation. These ligand-triggered protein-protein interactions are the central molecular actions of nuclear receptor-dependent 1α,25-$(OH)_2$D signalling.

An essential prerequisite for the direct modulation of transcription by 1α,25-$(OH)_2$D is the location of at least one activated VDR protein close to the transcription start site (TSS) of the respective primary 1α,25-$(OH)_2$D target gene. This is achieved through the specific binding of VDR to discrete DNA sequences in promoter regions of target genes, referred to as vitamin D response elements (VDREs). Most of the presently known natural VDREs are located within the first 1000 bp of the promoter sequence upstream of the TSS, with a consensus VDR core binding motif of RGKTSA. Simultaneous communication of individual promoter regions with the Pol II complex may occur through a discrete three-dimensional organisation of the promoter viaa large protein mediator complex. This arrangement allows the close contact of remote regions.

Vitamin D deficiency

Several high-risk groups for vitamin D deficiency have been identified including individuals who avoid sun exposure, living at high latitudes, having darkly pigmented skin, obese

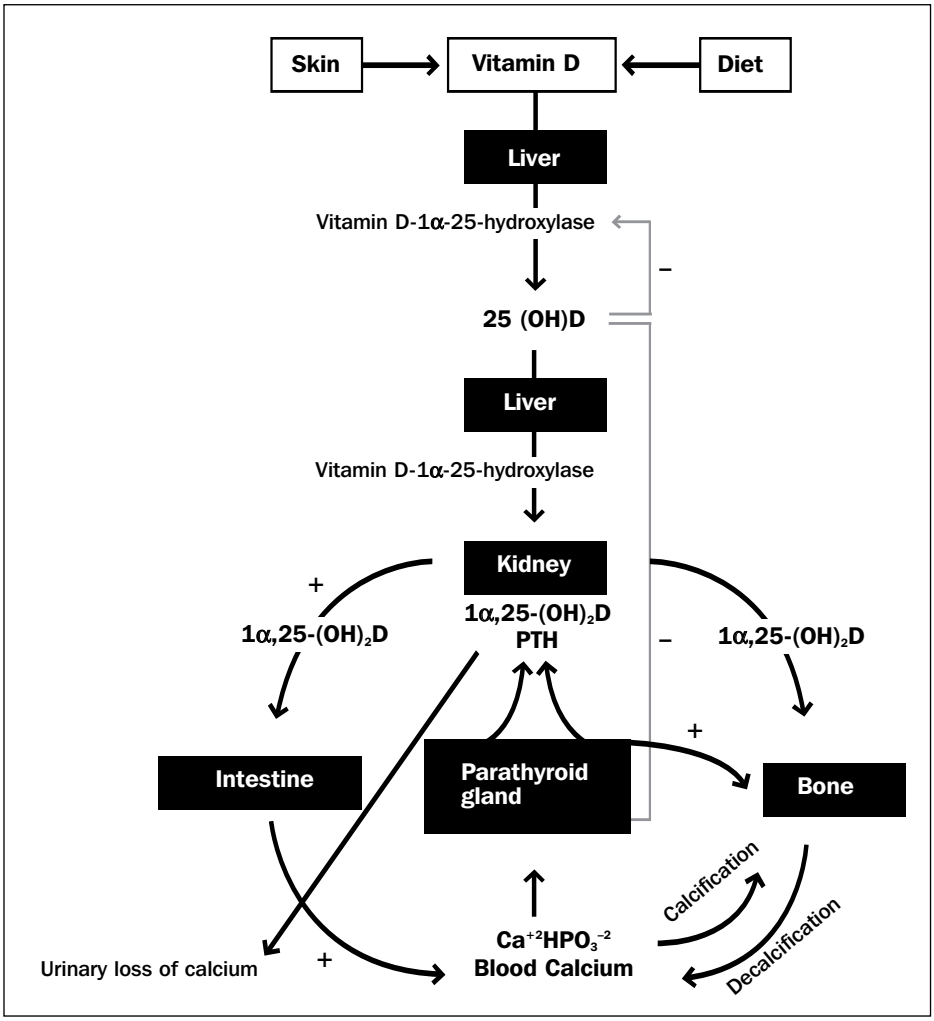

Fig. 2. The physiological role of PTH in the maintenance of serum calcium level. Key target organs for PTH – bone, kidney and intestine – and their feedback interactions with calcium are shown.

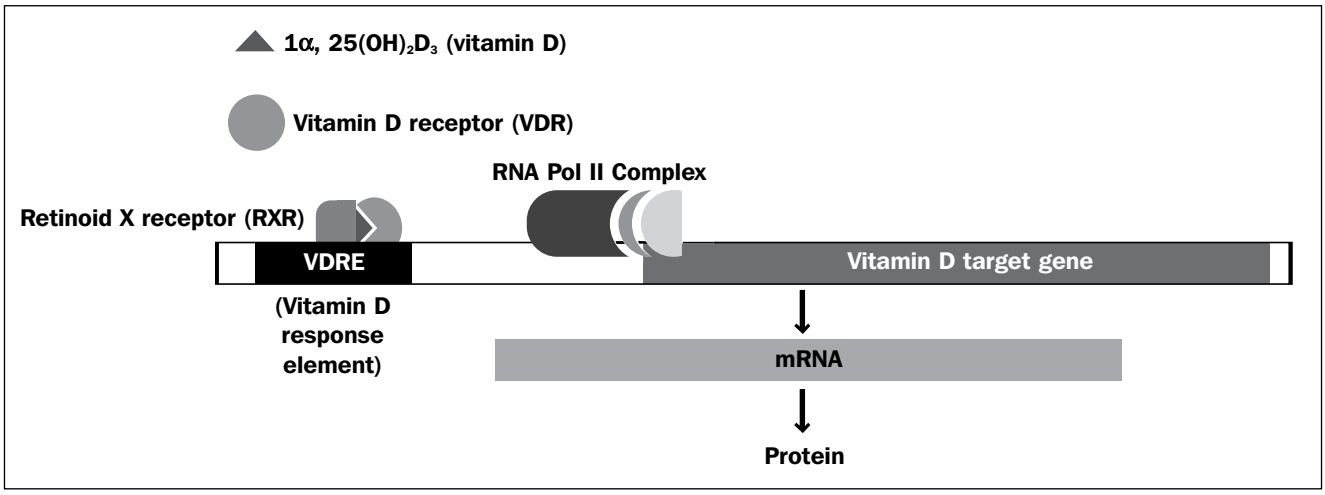

Fig. 3. Schematic representation of genomic action of vitamin D.

and those suffering from chronic kidney disease.[50] Serum 25-(OH)D concentration <50 nmol/L (< 20 ng/mL) has recently been defined as vitamin D deficiency.[50] Severe vitamin D deficiency is marked by a threshold <25 nmol/L (<10 ng/mL) and vitamin D insufficiency by concentration in the range 25–49 nmol/L (10–19 ng/mL) (Table 1).[51] At a concentration below 25 nmol/L (<10 ng/mL) adverse effects are observed in children and adults, and increased bone resorption and an elevated risk for secondary hyperthyroidism are seen at concentration of 25–49 nmol/L (10–19 ng/mL) (Fig. 4).

A 25-(OH)D threshold of 75 nmol/L (30 ng/mL) is needed for optimal bone mineral density in younger (19–49 years) and middle-aged adults (>50 years).[52] Increasing evidence suggests that 25-(OH)D serum concentration of 75–110 nmol/L (30–44 ng/mL) may have additional health benefits in reducing the risk of common cancers, autoimmune diseases, type 2 diabetes, cardiovascular disease and infectious disease (Fig. 2).[53–55]

Prevalence of vitamin D deficiency

Studies conducted on the German population estimated that the median concentration of 25-(OH)D in native children (1–17 age group) were 44 nmol/L. Another study, conducted in four Northern European countries including a total of 199 children with median age of 12.5 years, estimated that in 30–50% of the cases serum 25-(OH)D concentrations were below 25 nmol/L and in more than 90% of the cases serum 25-(OH)D concentrations were below 48nmol/L.[56]

In a Swiss study conducted on a large cohort comprising 3276 adults (25–75 age group), the median serum 25-(OH)D concentrations was 46 nmol/L, 34% had concentrations below 38 nmol/L, and about 70% had concentrations below 75 nmol/L.57 Similar data have been obtained from many other countries.[58–60]

van der Wielen and colleagues determined that 36% of older men and 47% of older women had 25-(OH)D serum concentrations below 30 nmol/L.[61] There is a high prevalence of vitamin D deficiency in the oldest segment of the US population.[52,59,62] Recently, a prevalence of vitamin D deficiency was determined in healthcare professional from the Qatari population and it was found that 96.5% had vitamin D <30 ng/mL and 20% had vitamin D levels below the detectable limit of <3 ng/mL.[63] This is an extremely high prevalence of vitamin D deficiency among young ambulatory individuals with no musculoskeletal complaints.

Consequences of vitamin D deficiency

The major function of vitamin D is to provide and maintain adequate calcium and phosphorus in the body to facilitate optimal metabolic function. Low vitamin D levels have been associated with a range of disorders. The association of low vitamin D and bone diseases such as rickets and osteoporosis is well known.[64,65] A positive association between vitamin D level and low bone mass in Saudi men and women has been shown recently.[66] In addition, vitamin D deficiency impairs reproductive success[67,68] and the ability to combat infections, in particular, tuberculosis, viral infections and influenza.[62,69,70] It may precipitate or worsen autoimmune conditions[71,72] and increase the incidence of death associated with heart disease,[73–75] stroke secondary to hypertension,[76] inflammatory bowel disease,[77] muscle weakness and falls,[52,78] fractures[79] and cancers of the breast, colon and prostate.[80–83]

Several lines of evidence have shown that vitamin D reduces the risk of colorectal cancer.[84–86] Other cancers that may be vitamin D-responsive include breast, lung, ovarian and prostate.[87] Other disorders in which the role of vitamin D is being actively investigated are the autoimmune disorders such as multiple sclerosis (MS), type 1 diabetes mellitus and rheumatoid arthritis.[88,89] The effect of vitamin D on asthma pathogenesis and control has also been extensively investigated.[90–92]

Vitamin D measurement

The two forms of vitamin D metabolite, 1α,25-$(OH)_2$D and 25-(OH)D are commonly measured in serum and have been the target of most genetic studies focusing on vitamin D metabolism. However, 25-(OH)D serum concentration is widely accepted as the best indicator of the vitamin D status of an individual *in vivo*. It covers not only the endocrine but also the paracrine biological pathways of vitamin D, whereas the active hormone 1α,25-$(OH)_2$D does not provide

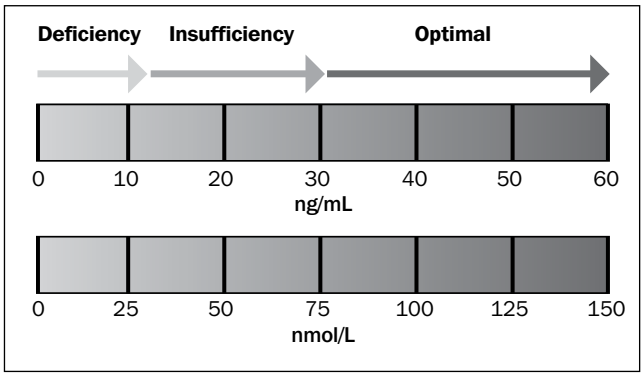

Fig. 4. Schematic representation of vitamin D deficiency, insufficiency and optimal ranges.

information on the vitamin D status and is often normal or increased as a result of secondary hyperthyroidism associated with vitamin D deficiency.[93] 25-(OH)D has an almost 1000-fold greater concentration than 1α,25-$(OH)_2D$; also, 25-(OH)D has a longer half-life (20 days) and hence is more stable in the circulation. Therefore, total-body vitamin D stores are best measured by assessing circulating levels of 25-(OH)D.[94]

There is a wide range of different immunological, mass-spectrometry-based and spectrophotometric methods currently used to measure serum 25-(OH)D concentration.[95–97] However, a number of studies have found considerable variability in results of 25-(OH)D measurements between analytical methods. This includes methods based on radioimmunochemistry, high-performance liquid chromatography with UV detection (HPLC-UV) or isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC/tandem MS).[96,98–101] Huge discrepancy has been known for many years between the methods, which can lead to misdiagnosis of patients and misinterpretation of population data.[102–107]

Vitamin D pathway gene polymorphisms and association with disease

Vitamin D, its receptor and the genes involved in vitamin D synthesis pathway have been studied intensively due to an association with various diseases of public health importance,

VDR

The *VDR* gene is located on chromosome 12q13 and consists of nine exons. It encodes a nuclear hormone receptor, VDR, which is an intracellular receptor belonging to the steroid/thyroid nuclear receptor family. It is the specific receptor for vitamin D, an immunomodulator through which vitamin D exerts its effects. The VDR protein contains the DNA-binding sites encoded by exon 2 and 3 and the ligand-binding site encoded by exon 4 to 9.

To date, a number of VDR polymorphisms have been discovered that are located in the promoter, in and around exons 2 to 9 and in the 3′UTR region. Analysis of the importance of these VDR polymorphisms for various diseases has proved difficult. As a result, only a few polymorphisms of this gene have been studied. Studies examining VDR polymorphisms reported significant associations with diabetes, arthritis, autoimmune diseases and essential hypertension.[108–111] Significant associations between polymorphisms in the *VDR* gene with asthma have been reported in several genetic association studies,[112,113] but has not been replicated.[114,115] The SNP (7968585) in the *VDR* gene has been reported to be significantly associated with the Parkinson's disease in patients from two different cohorts.[116,117] Feng and colleagues showed significant association of autoimmune thyroid diseases (AITD) with the VDR gene polymorphisms TaqI (rs731236) and BsmI (rs1544410).[118]

CYP2R1

The synthesis of the most active vitamin D metabolite, 1·,25-(OH)2D3, requires two hydroxylations, one at the 25 and one at the 1α positions. In the liver, CYP2R1 (25-hydroxylase) catalyses vitamin D3 to 25-hydroxyvitamin D3 (25-[OH]D_3), the main circulating vitamin D metabolite, while CYP27B1 (1a-hydroxylase) catalyses 25(OH)D3 to1α, $25(OH)_2D_3$ in the kidney.

The *CYP2R1* gene is located on chromosome 11p15.2, contains five exons and spans about 15.5 kb. It catalyses the synthesis of $25(OH)D_3$ in the liver. It has been reported that the GG genotype of CYP2R1 polymorphism (rs10741657) increases the risk of developing of type 1 diabetes in two ethnically different populations.[119,120]

One SNP, rs10741657, which resides in the 5′ region of the *CYP2R1* gene, was associated with serum 25-(OH)D concentrationsin a transmission disequilibrium test (TDT) study in a German population.[121] The rs10877012 C allele was associated with lower levels of 25-(OH)D in a study of gestational diabetic patients.[122] The effect of the rs10877012 C allele on lower levels of 25-(OH)D was also reported in African-Americans recently.[123] Although the effect of rs10877012 on 25-(OH)D levels has been replicated in candidate gene studies, there is no report on how this single-nucleotide polymorphism (SNP) modulates 25-(OH)D levels in serum. However, CYP27B1 functions downstream of circulating 25-(OH)D. Therefore, rs10877012, or the causal SNP captured by this SNP, could possibly alter the role of CYP27B1 in metabolic feedback loops or adjust the rate at which 25-(OH)D is metabolised.[124]

CYP27B1

The *CYP27B1* gene is located on chromosome 12q13.1-13.3 spanning 6.66 kb on the reverse strand. It catalyses the synthesis of $1,25(OH)_2D_3$ from $25(OH)D_3$ in the kidney. The CC genotype of CYP27B1 polymorphism (rs10877012) increases the risk of developing of type 1 diabetes in Egyptian and UK populations.[120,125] Moreover, it has been found that there is a synergism between the GG genotype of *CYP2R1* and CC genotype of *CYP27B1* regarding the risk of development of type 1 diabetes, and the level of vitamin D and calcium is significantly lower in individuals with the GG genotype of *CYP2R1* and CC genotype of *CYP27B1*.[120] The possible explanation for this is that the *CYP2R1* polymorphism could change the enzyme activity and subsequently cause a relative lack of $25(OH)D_3$, the substrate for the active form of vitamin D. This may explain the low $25(OH)D_3$ serum levels in individuals with the *CYP2R1* GG allele.[121]

The presence of the *CYP27B1* CC allele significantly reduces mRNA levels,[121] and thereby reduces the level of the

active1α-hydroxylase and conversion of 25(OH)D to 1α,25$(OH)_2$D, causing low concentrations of 1,25$(OH)_2$D,[126, 127] which lead to increased predisposition to type 1 diabetes.[125] The associations of two other *CYP27B1* SNPs, rs4646536 and rs703842, with 25-(OH)D level were reported in a Canadian multiple sclerosis study.[124] However, associations of these two SNPs were not observed in Hispanics and African-Americans.[128] The inconsistency of this association may be due to tight regulation of circulating 1α,25-$(OH)_2$D concentrations through 1α-hydroxylation, the relatively small sample sizes in these studies, or the different ethnicities.[129]

CYP24A1A

The *CYP24A1A* gene is the third cytochrome P-450 gene involved in regulation of vitamin D concentration. It encodes the 1α,25$(OH)_2$D inactivation protein. The *CYP24A1A* gene is located on chromosome 20, at 20q13.2-q13.3, spanning 20.53 kb on the reverse strand. An intronic SNP, rs17219315, was associated with 25(OH)D levels in a family-based study using TDT. This SNP was included in another association study on *CYP24A1* and serum 25(OH)D concentration, in which no significant findings were reported for this gene.[130]

Vitamin D as an anticancer agent

Multiple studies have confirmed that vitamin D induces growth arrest, triggers cell death and/or promotes differentiation of cancer cells *in vitro* and established tumours *in vivo*. Experimental evidence suggests that vitamin D may reduce the risk of cancer through regulation of cellular proliferation and differentiation and inhibition of angiogenesis.[131] In cancer cells, the active metabolite of vitamin D, 1α,25 $(OH)_2D_3$, suppresses cell proliferation.[132] Extensive research has shown that cells, including cancer cells, express VDRs. Binding of vitamin D to its receptor regulate more than 60 genes that exert prodifferentiating, antiproliferative and antimetastatic effects on cells, including effects on the ell cycle.[133]

The overall role of vitamin D in cancer also is largely supported by epidemiological observations. Several ecological studies suggest that sunlight may protect against prostate, colon, rectal, breast and ovarian cancer. Cancer incidence specifically for that of colon and breast is lower geographically where there is increased sunlight.[134] Some analytical studies also suggest a protective association between circulating vitamin D in blood and colorectal and prostate cancer.[135] Similarly, plasma levels of 25$(OH)D_3$ are also well correlated with the disease. Studies showed that 87% of the triple negative breast cancer patients had inadequate levels of serum 25$(OH)D_3$. Mortality rate from prostate cancer is inversely related to the level of UV radiation, the major source of vitamin D.[133] Two separate retrospective analyses showed that mean vitamin D levels were lower in subjects who later developed prostate cancer compared with age-matched controls.[136] Similarly, a 67% lower risk of colorectal cancer was found among the women in the highest quintile of consistent vitamin D intake over time.[135,137]

One of the recent reports indicated that serum concentration of 130 nmol/L or approximately 50 ng/mL protected against breast cancer by nearly 50%.[138] Similarly, there have been several correlative studies that have indicated a positive correlation of serum vitamin D levels and protection from colon and prostate cancers.[139] These results provide a rationale for using vitamin D for cancer prevention or therapy.

Vitamin D and infertility

Vitamin D deficiency might be important for endocrine disturbances including fertility in women as well as in men. VDR mRNA has been shown to be expressed in the ovaries, in mixed ovarian cells, and in purified granulosa cell cultures, indicating a role in steroidogenesis of sex hormones.[140,141] Likewise, the placenta expresses the *CYP27B1* gene (encoding 1α-hydroxylase) and *VDR* gene.[142,143]

In male rodents, VDR has been found in the smooth muscle of the epididymis, spermatogonia and Sertoli cells.[144,145] Recently, VDR was detected in human sperm, specifically in the sperm nucleus.[146,147] More recently, it was reported that spermatids, epididymis, seminal vesicle and prostate express VDR and vitamin D metabolizing enzymes.[148] A 45% reduction in successful matings and up to 73% decreased overall fertility rate has been found in vitamin D-deficient male rats.[149] The testes of vitamin D-deficient rats showed incomplete spermatogenesis and degenerative changes.[150]

The overall fertility is reduced in vitamin D-deficient diet eating female rats and have increased risk of pregnancy complications. This is not corrected by normalising the hypocalcemia in vitamin D-deficient female rats, but requires 1,25$(OH)_2D_3$.[149] Vdr knockout female mice conceive infrequently, have significantly fewer viable fetuses *in utero* and present with uterine hypoplasia, impaired folliculogenesis, anovulation, and absent corpora lutea.[21, 151–153] In Vdr null mutant mice, feeding high calcium diets partly restores fertility and increases the rate of conception but does not normalise the number or weight of viable fetuses.[21,154] Vdr null male mutant mice show significant gonadal insufficiency, with decreased sperm count and motility, and histological abnormalities of the testis.[154]

Several studies suggest an association between vitamin D and fertility in humans. Evidence exists that vitamin D exerts some effects on female reproduction, including IVF outcome, PCOS and endometriosis, as well as on steroidogenesis in healthy women.[155] In a study among 84 infertile women undergoing IVF, women with higher levels of 25(OH)D in serum and follicular fluid were significantly more likely to achieve clinical pregnancy following IVF, and high vitamin D levels were significantly associated with improved parameters of controlled ovarian hyperstimulation.[156] In men, vitamin D status might be related to spermatogenesis, semen quality and testiculopathies as well as male hypogonadism.[155] A positive correlation of 25(OH)D serum levels with sperm motility and progressive motility has been found in a cross-sectional study including 300 men from the general population.[157] Moreover, men with vitamin D deficiency (<10 ng/mL) had a lower proportion of motile, progressive motile, and morphologically normal spermatozoa compared with men with sufficient vitamin D status (>30 ng/mL).

Vitamin D deficiency in diabetes mellitus

Many epidemiological studies have found high prevalence of vitamin D deficiency in children with type 1 diabetes mellitus (TIDM), which suggests that an association exists between vitamin D deficiency and T1DM.[158] Type 1 diabetes mellitus is an autoimmune disorder and it has been postulated that immune modulatory actions of vitamin D decrease the cytokine production and lymphocyte proliferation, thus preventing destruction of β-cells and subsequent development of type 1 diabetes mellitus.[159–162] An inverse relationship has also been established between low intake of total vitamin D and risk of type 2 diabetes mellitus (T2DM).[163–165] An association between *VDR* polymorphism and T1DM was reported in Indian, German, and Taiwan populations.[166–168] Also, polymorphism in the *CYP27B1* gene has been shown to decrease the local expression of 1-alpha-hydroxylase, and subsequent decreased conversion of 25(OH)D to 1α, $25(OH)_2D$, resulting in increased predisposition to T1DM.

Vitamin D and asthma

Research focused on identification of genetic variants that could influence vitamin D levels has been minimal until recently. Specific candidate genes have been studied in relation to vitamin D using relatively small and underpowered studies.[121,128] Recently, three genome-wide association studies (GWAS) of vitamin D have been published, with the largest study using 15 cohorts of 33,996 individuals.[74,169] In that study, Wang and colleagues[74] identified several genetic variants for vitamin D level including rs2282679 located in the *GC* gene (which encodes vitamin D carrier protein) on chromosome 4p12; rs12785878 located near the *DHCR7* gene on chromosome 11q12, and rs10741657 located near the *CYP2R1* gene on 11p15.[74]

Lasky-Su and colleagues[170] identified four SNPs associated with serum vitamin D in two independent cohorts of asthmatic children. These variants were in/near four genes including rs11002969 located in an intronic region of the *ZCCHC24* gene on chromosome 10q22.3 and has previously been associated with right ventricular cardiomyopathy; rs163221 is located in an intronic region of the *C18orf16/CHST9* gene (carbohydrate *N*-acetyl galactosamine sulphotransferase 9); rs1678849 is located in anintronic region of the *FUT6* gene; and rs4864976 is located on chromosome 4q12 and is not proximal to any relevant candidate gene. None of these SNPs or the nearby genes has a known clear involvement in biologic pathways related to vitamin D. The SNP rs2282679 which has been significantly associated with circulating vitamin D levels was also supported by a well-powered GWAS on insufficient vitamin D levels.[74] This association was further conformed by Lasky-Su and colleagues.[170] This supports the notion that combined association from the above three asthma cohorts is significant at a genome-wide level. Significant associations between polymorphisms in the *VDR* gene with asthma have been reported in several genetic association studies, but has not been consistently replicated.[112–114]

Future prospects

Vitamin D plays an important role in diverse physiological functions. Vitamin D promotes calcium and phosphorus absorption, which is necessary to build and maintain bones and teeth, and is also a transcription factor in most cells in the body. $1\alpha,25\text{-}(OH)_2D$/VDR complex triggers global changes in gene expression via classical transcriptional mechanisms that contribute to induction of quiescence and maintenance of the differentiated phenotype in epithelial cells. In addition, novel mechanisms of vitamin D signalling have been identified, including regulation of miRNAs, rapid signalling through kinase pathways and protein-protein interactions. The demonstration that vitamin D metabolites and analogs that do not activate VDR-mediated transcription can mimic some of the anti-tumour actions of $1\alpha,25\text{-}(OH)_2D$ indicates that additional mechanisms of action remain to be discovered.

Receptors for vitamin D were found in a variety of cells and tissues. Therefore, the likely disturbances due to vitamin D deficiency and the therapeutic potential of vitamin D are expanding. In spite of the substantial advancement of our understanding of the metabolism of vitamin D, the distribution of $1\alpha,25\text{-}(OH)_2D$ and 25-(OH)D in body pools, including their storage and mobilization, is notably lacking.

Measuring circulating level of 25(OH)D provides a clue about a person's vitamin D deficiency/insufficiency. There are a variety of assays used to measure 25(OH)D. The radioimmunoassay and competitive protein binding assays for 25(OH)D are useful in detecting vitamin D deficiency and sufficiency. However, these assays have technical difficulties especially if they are not run routinely. Several reference laboratories have now switched to LC-MS, which measures both $25(OH)D_2$ and $25(OH)D_3$ quantitatively. The total 25(OH)D (i.e., $25(OH)D_2$ plus $25(OH)D_3$ is what physicians need to be aware of for their patients. A level >30 ng/mL is now considered to be the preferred healthy level that all children and adults should maintain throughout the year.

Vitamin D deficiency is a worldwide health problem. Vitamin D insufficiency has become a common problem for many individuals and is now linked to various diseases. Serum 25(OH)D levels are inversely associated with overweight, abdominal obesity, metabolic syndrome, systolic blood pressure and stroke, and plasma glucose concentrations. Vitamin D deficiency is associated with secondary hyperparathyroidism, higher systolic blood pressure, lower serum calcium, lower high-density lipoprotein levels, and increased incidence of insulin resistance. Moreover, lower serum 25(OH)D levels are associated with increased morbidity and mortality, all-cause mortality, myocardial infarction and diabetes. Normalisation of vitamin D reverses some of these negative phenomena.

Several studies on vitamin D level in different countries have demonstrated the magnitude of the problem. Vitamin D deficiency is common in the Middle East. A low vitamin D level in the Saudi Arabian population has been reported despite the fact that sunny days are almost all year round. Recently, it has been found that 25–37% of the healthy Saudi men have vitamin D deficiency. Although the optimal concentration for overall health is currently under debate, lower levels of vitamin D have been associated with a greater risk of rickets in children or osteomalacia in adults,

increased risk of fractures, falls, breast cancer, colorectal cancer and adenoma, poor immunity and cardiovascular and other diseases such as multiple sclerosis. This therefore has led to substantial interest in identifying determinants of vitamin D. As many of environmental determinants of vitamin D are known, identifying genetic determinants of vitamin D is likely to help in our overall understanding of the biological processes that may increase or decrease vitamin D levels.

Sunlight exposure often is limited by lifestyle and other choices, making it difficult to obtain enough vitamin D from diet alone; thus, patients with deficiency are likely to require long-term supplementation. Considering the available facts, the introduction of a national policy to provide routine supplementation of vitamin D to vulnerable populations not only would reduce various morbidities, falls and fractures, but also would eliminate other morbidities with minimal cost. Extra vitamin D should be provided to premature infants and those who are exclusively breast-fed.

In conclusion, vitamin D deficiency/insufficiency is a global public health problem not only limited to high risk groups of elderly or the housebound, but also young physicians and nurses have an alarmingly high prevalence of vitamin D deficiency. This review highlights the findings that confirm the widespread prevalence of vitamin D deficiency and the need for a population-wide policy to solve this problem. Moreover, further genomic investigations in larger groups as well as functional studies need to performed to confirm previous findings. □

References

1 Pedersen JI. Vitamin D requirement and setting recommendation levels – current Nordic view. *Nutr Rev* 2008; **66**: S165–9.

2 Mosekilde L. Vitamin D requirement and setting recommendation levels: long-term perspectives. *Nutr Rev* 2008; **66**: S170–7.

3 Mehta RG, Peng X, Alimirah F, Murillo G, Mehta R. Vitamin D and breast cancer: emerging concepts. *Cancer Lett* 2012; **12**: 639–8.

4 Welsh J. Vitamin D and cancer: integration of cellular biology, molecular mechanisms and animal models. *Scand J Clin Lab Invest Suppl* 2012; **243**: 103–11.

5 Cooke NE, Haddad JG. Vitamin D binding protein (Gcglobulin). *Endocr Rev* 1989; **10**: 294–307

6 Bikle DD, Gee E, Halloran B, Haddad JG. Free 1,25-dihydroxyvitamin D levels in serum from normal subjects, pregnant subjects, and subjects with liver disease. *J Clin Invest* 1984; **74**: 1966–71.

7 Bikle DD, Siiteri PK, Ryzen E, Haddad JG. Serum protein binding of 1,25-dihydroxyvitamin D: a reevaluation by direct measurement of free metabolite levels. *J Clin Endocrinol Metab* 1985; **61**: 969–75.

8 Haddad JG, Matsuoka LY, Hollis BW, Hu YZ, Wortsman J. Human plasma transport of vitamin D after its endogenous synthesis. *J Clin Invest* 1993; **91**: 2552–5.

9 Haddad JG. Plasma vitamin D-binding protein (Gc-globulin): multiple tasks. *J Steroid Biochem Mol Biol* 1995; **53**: 579–82.

10 Cheng JB, Motola DL, Mangelsdorf DJ, Russell DW. De-orphanization of cytochrome P450 2R1: a microsomal vitamin D 25-hydroxilase. *J Biol Chem* 2003; **278**: 38084–93.

11 Ohyama Y, Yamasaki T. Eight cytochrome P450s catalyze vitamin D metabolism. *Front Biosci* 2004; **9**: 3007–18.

12 Strushkevich N, Usanov SA, Plotnikov AN, Jones G, Park HW. Structural analysis of CYP2R1 in complex with vitamin D3. *J Mol Biol* 2008; **380**: 95–106.

13 Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. *Proc Natl Acad Sci USA* 2004; **101**: 7711–5.

14 St-Arnaud R, Messerlian S, Moir JM, Omdahl JL, Glorieux FH. The 25-hydroxyvitamin D 1-alpha-hydroxylase gene maps to the pseudovitamin D-deficiency rickets (PDDR) disease locus. *J Bone Miner Res* 1997; 12: 1552–9.

15 Zehnder D, Bland R, Williams MC *et al*. Extrarenal expression of 25-hydroxyvitamin d3–1 alpha-hydroxylase. *J Clin Endocrinol Metab* 2001; **86**: 888–94.

16 Flanagan JN, Wang L, Tangpricha V, Reichrath J, Chen TC, Holick MF. Regulation of the 25-hydroxyvitamin D-1alpha-hydroxylase gene and its splice variant. *Recent Results Cancer Res* 2003; **164**: 157–67.

17 Wang L, Whitlatch LW, Flanagan JN, Holick MF, Chen TC. Vitamin D autocrine system and prostate cancer. *Recent Results Cancer Res* 2003; **164**: 223–7.

18 Fritsche J, Mondal K, Ehrnsperger A, Andreesen R, Kreutz M. Regulation of 25-hydroxyvitamin D3–1alpha-hydroxylase and production of 1alpha,25-dihydroxyvitamin D3 by human dendritic cells. *Blood* 2003; **102**: 3314–6

19 DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004; **80**: 1689S–96S.

20 Kogawa M, Findlay DM, Anderson PH *et al*. Osteoclastic metabolism of 25(OH)-vitamin D3: a potential mechanism for optimization of bone resorption. *Endocrinology* 2010; **151**: 4613–25

21 Panda DK, Miao D, Tremblay ML *et al*. Targeted ablation of the 25-hydroxyvitamin D 1alpha-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. *Proc Natl Acad Sci USA* 2001; **98**: 7498–503.

22 Dardenne O, Prud'homme J, Arabian A, Glorieux FH, St-Arnaud R. Targeted inactivation of the 25-hydroxyvitamin D(3)-1(alpha)-hydroxylase gene (CYP27B1) creates an animal model of pseudovitamin D-deficiency rickets. *Endocrinology* 2001; **142**: 3135–41.

23 Kawashima H, Torikai S, Kurokawa K. Localization of 25-hydroxyvitamin D3 1alpha-hydroxylase and 24-hydroxylase along the rat nephron. *Proc Natl Acad Sci USA* 1981; **78**: 1199–203.

24 Zierold C, Darwish HM, DeLuca HF. Identification of a vitamin D-response element in the rat calcidiol (25-hydroxyvitamin D3) 24-hydroxylase gene. *Proc Natl Acad Sci USA* 1994; **91**: 900–2.

25 Zehnder D, Bland R, Walker EA *et al*. Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in the human kidney. *J Am Soc Nephrol* 1999; **10**: 2465–73.

26 Makin G, Lohnes D, Byford V, Ray R, Jones G. Target cell metabolism of 1,25-dihydroxyvitamin D3 to calcitroic acid. Evidence for a pathway in kidney and bone involving 24-oxidation. *Biochem J* 1989; **262**: 173–80.

27 Reddy GS, Tserng KY. Calcitroic acid, end product of renal metabolism of 1,25-dihydroxyvitamin D3 through C-24 oxidation pathway. *Biochemistry* 1989; **28**: 1763–9.

28 St-Arnaud R. Novel findings about 24,25-dihydroxyvitamin D: an active metabolite? *Curr Opin Nephrol Hypertens* 1999; **8**: 435–41.

29 Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; **357**: 266–81.

30 Jenster G, Spencer TE, Burcin MM, Tsai SY, Tsai MJ, O'Malley BW. Steroid receptor induction of gene transcription: a two-step model. *Proc Natl Acad Sci USA* 1997; **94**: 7879–84.

31 Bouillon R, Carmeliet G, Verlinden L *et al*. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev* 2008; **29**: 726–76.

32 Haussler MR, Whitfield GK, Haussler CA *et al*. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J Bone Miner Res* 1998; **13**: 325–49.

33 Norman AW. Mini review: vitamin D receptor: new assignments for an already busy receptor. *Endocrinology* 2006; **147**: 5542–8.

34 Freedman LP, Arce V, Perez Fernandez R. DNA sequences that act as high affinity targets for the vitamin D3 receptor in the absence of the retinoid X receptor. *Mol Endocrinol* 1994; **8**: 265–73.

35 Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. *Physiol Rev* 1998; **78**: 1193–231.

36 Smolders J, Peelen E, Thewissen M *et al*. The relevance of vitamin D receptor gene polymorphisms for vitamin D research in multiple sclerosis. *Autoimmun Rev* 2009; **8**: 621–6.

37 Dowd DR, MacDonald PN. The 1,25-dihydroxyvitamin D3-independent actions of the vitamin D receptor in skin. *J Steroid Biochem Mol Biol* 2010; **121**: 317–21.

38 Zella LA, Meyer MB, Nerenz RD, Lee SM, Martowicz ML, Pike JW. Multifunctional enhancers regulate mouse and human vitamin D receptor gene transcription. *Mol Endocrinol* 2010; **24**: 128–47.

39 Omdahl JL, Bobrovnikova EA, Choe S, Dwivedi PP, May BK. Overview of regulatory cytochrome P450 enzymes of the vitamin D pathway. *Steroids* 2001; **66**: 381–9.

40 Carlberg C, Polly P. Gene regulation by vitamin D 3. *Crit Rev Eukaryot Gene Expr* 1998; **8**: 19–42.

41 Shaffer PL, Gewirth DT. Structural basis of VDR-DNA interactions on direct repeat response elements. *EMBO J* 2002; **21**: 2242–52.

42 Rochel N, Wurtz JM, Mitschler A, Klaholz B, Moras D. The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand. *Mol Cell* 2000; **5**: 173–9.

43 Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev* 2000; **14**: 121–41.

44 Polly P, Herdick M, Moehren U, Baniahmad A, Heinzel T, C arlberg C. VDR-Alien: a novel, DNA-selective vitamin D 3 receptor-corepressor partnership. *FASEB J* 2000; **14**: 1455–63.

45 Leo C, Chen JD. The SRC family of nuclear receptor coactivators. *Gene* 2000; **245**: 1–11

46 Kwok RP, Lundblad JR, Chrivia JC *et al*. Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature 1994; **370**: 223–6

47 Castillo AI, Jimenez-Lara AM, Tolon RM, Aranda A. Synergistic activation of the prolactin promoter by vitamin D receptor and GHF-1: role of coactivators, CREB-binding protein and steroid hormone receptor coactivator-1 (SRC-1). *Mol Endocrinol* 1999; **13**: 1141–54

48 Rachez C , Suldan Z , Ward J *et al*. A novel protein complex that interacts with the vitamin D 3 receptor in a ligand-dependent manner and enhances transactivation in a cell-free system. *Genes Dev* 1998; **12**: 1787–800.

49 Rachez C, Lemon BD, Suldan Z *et al*. Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. *Nature* 1999; **398**: 824–8.

50 Ross AC, Manson JE, Abrams SA *et al*. Clarification of DRIs for calcium and vitamin D across age groups. *J Am Diet Assoc* 2011; **111**: 1467.

51 Holick MF, Binkley NC, Bischoff-Ferrari HA *et al*.; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011; **96**: 1911–30.

52 Bischoff-Ferrari HA, Dietrich T, Orav EJ *et al.* Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y. *Am J Clin Nutr* 2004; **80**: 752–8.

53 Forman JP, Giovannucci E, Holmes MD *et al*. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* 2007; **49**: 1063–9.

54 Giovannucci E. Epidemiological evidence for vitamin D and colorectal cancer. *J Bone Miner Res* 2007; **2**: V81–5.

55 Pilz S, Tomaschitz A, Obermayer-Pietsch B, Dobnig H, Pieber TR. Epidemiology of vitamin D insufficiency and cancer mortality. *Anticancer Res* 2009; **29**: 3699–704.

56 Tylavsky FA, Cheng S, Lyytikäinen A, Viljakainen H, Lamberg-Allardt C. Strategies to improve vitamin D status in northern European children: exploring the merits of vitamin D fortification and supplementation. *J Nutr* 2006; **136**: 1130–4.

57 Burnand B, Sloutskis D, Gianoli F *et al*. Serum 25-hydroxyvitamin D: distribution and determinants in the Swiss population. *Am J Clin Nutr* 1992; **56**: 537–42.

58 McKenna MJ. Differences in vitamin D status between countries in young adults and the elderly. *Am J Med* 1992; **93**: 69–77.

59 Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF, Yetley EA. Serum 25-hydroxyvitamin D status of the US population: 1988-1994 compared with 2000-2004. *Am J Clin Nutr* 2008; **88**: 1519–27.

60 van Schoor NM, Lips P. Worldwide vitamin D status. *Best Pract Res Clin Endocrinol Metab* 2011; **25**: 671–80.

61 van der Wielen RP, Löwik MR, van den Berg H *et al*. Serum vitamin D concentrations among elderly people in Europe. *Lancet* 1995; **346**: 207–10.

62 Ginde AA, Mansbach JM, Camargo CA Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 2009; **169**: 384–90.

63 Mahdy S, Al-Emadi SA, Khanjar IA *et al*. Vitamin D status in health care professionals in Qatar. *Saudi Med J* 2010; **31**: 74–7.

64 Heaney RP. Vitamin D, nutritional deficiency, and the medical paradigm. *J Clin Endocrinol Metab* 2003; **88**: 5107–8.

65 Heaney RP. The Vitamin D requirement in health and disease. *J Steroid Biochem Mol Biol* 2005; **97**: 13–9.

66 Sadat-Ali M, Al Elq AH, Al-Turki HA, Al-Mulhim FA, Al-Ali AK. Influence of vitamin D levels on bone mineral density and osteoporosis. *Ann Saudi Med* 2011; **31**: 602–8.

67 Bodnar LM, Catov JM, Zmuda JM *et al*. Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. *J Nutr* 2010; **140**: 999–1006.

68 Lewis S, Lucas RM, Halliday J *et al*. Vitamin D deficiency and pregnancy: from preconception to birth. *Mol Nutr Food Res* 2010; **54**: 1092–102.

69 Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. *Int J Epidemiol* 2008; **37**: 113–9.

70 Chesney RW. Vitamin D and The Magic Mountain: the antiinfectious role of the vitamin. *J Pediatr* 2010; **156**: 698–703.

71 Zhang HL, Wu J. Role of vitamin D in immune responses and autoimmune diseases, with emphasis on its role in multiple sclerosis. *Neurosci Bull* 2010; **26**: 445–54.

72 Blaney GP, Albert PJ, Proal AD. Vitamin D metabolites as clinical markers in autoimmune and chronic disease. *Eur J Clin Invest* 2005; **35**: 290–304.

73 Semba RD, Houston DK, Bandinelli S *et al*. Relationship of 25-hydroxyvitamin D with all-cause and cardiovascular disease mortality in older community-dwelling adults. *Eur J Clin Nutr* 2010; **64**: 203–9.

74 Wang L, Manson JE, Song Y *et al*. Systematic review: vitamin D and calcium supplementation in prevention of cardiovascular events. *Ann Intern Med* 2010; **152**: 315–23.

75 Drechsler C, Pilz S, Obermayer-Pietsch B *et al*. Vitamin D deficiency is associated with sudden cardiac death, combined cardiovascular events, and mortality in haemodialysis patients. *Eur Heart J* 2010; **31**: 2253–61.

76 Pilz S, Tomaschitz A, Drechsler C *et al*. Vitamin D supplementation: a promising approach for the prevention and treatment of strokes. *Curr Drug Targets* 2011; **12**: 88–96.

77 Levin AD, Wadhera V, Leach ST *et al*. Vitamin D deficiency in children with inflammatory bowel disease. *Dig Dis Sci* 2011; **56**: 830–6.

78 Pfeifer M, Begerow B, Minne HW. Vitamin D and muscle function. *Osteoporos Int* 2002; **13**: 187–94.

79 Wimalawansa SJ. *Vitamin D: Everything You Need to Know*. Homagama, Sri Lanka: Karunaratne, 2011.

80 Hisatake J, O'Kelly J, Uskokovic MR *et al*. Novel vitamin D(3) analog, 21-(3-methyl-3-hydroxy-butyl)-19-nor D(3), that modulates cell growth, differentiation, apoptosis, cell cycle, and induction of PTEN in leukemic cells. *Blood* 2001; **97**: 2427–33.

81 Morales-Tirado V, Wichlan DG, Leimig TE *et al*. 1alpha,25-dihydroxyvitamin D3 (vitamin D3) catalyzes suppressive activity on human natural regulatory T cells, uniquely modulates cell cycle progression, and augments FOXP3. *Clin Immunol* 2011; **138**: 212–21.

82 Jenab M, Bueno-de-Mesquita HB, Ferrari P *et al*. Association between pre-diagnostic circulating vitamin D concentration and risk of colorectal cancer in European populations:a nested case-control study. *BMJ* 2010; **340**: b5500.

83 Karlsson S, Olausson J, Lundh D *et al*. Vitamin D and prostate cancer: the role of membrane initiated signaling pathways in prostate cancer progression. *J Steroid Biochem Mol Biol* 2010; **121**: 413–6.

84 Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). *Cancer Causes Control* 2005; **16**: 83–95.

85 Giovannucci E. The epidemiology of vitamin D and colorectal cancer: recent findings. *Curr Opin Gastroenterol* 2006; **22**: 24–9.

86 Gorham ED, Garland CF, Garland FC *et al.* Vitamin D and prevention of colorectal cancer. *J Steroid Biochem Mol Biol* 2005; **97**: 179–94.

87 Grant WB. The likely role of vitamin D from solar ultraviolet-B irradiance in increasing cancer survival. *Anticancer Res* 2006; **26**: 2605–14.

88 Hyppönen E. Vitamin D and increasing incidence of type 1 diabetes-evidence for an association? *Diabetes Obes Metab* 2010; **12**: 737–43

89 Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG; Iowa Women's Health Study. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. *Arthritis Rheum* 2004; **50**: 72–7.

90 Brehm JM, Celedón JC, Soto-Quiros ME *et al*. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med* 2009; **179**: 765–71.

91 Brehm JM, Schuemann B, Fuhlbrigge AL *et al.*; Childhood Asthma Management Program Research Group. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol* 2010; **126**: 52–8.

92 Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? *J Allergy Clin Immunol* 2007; **120**: 1031–5.

93 Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol* 2009; **19**: 73–8.

94 Lips P. Relative value of 25(OH)D and 1,25(OH)2D measurements. *J Bone Miner Res* 2007; **22**: 1668–71.

95 Jones G, Horst R, Carter G, Makin HL. Contemporary diagnosis and treatment of vitamin D-related disorders. *J Bone Miner Res* 2007; **2**: V11–15.

96 Wallace AM, Gibson S, de la Hunty A, Lamberg-Allardt C, Ashwell M. Measurement of 25-hydroxyvitamin D in the clinical laboratory: current procedures, performance characteristics and limitations. *Steroids* 2010; **75**: 477–88.

97 Carter GD. Accuracy of 25-hydroxyvitamin D assays: confronting the issues. *Curr Drug Targets* 2011; **12**: 19–28.

98 Binkley N, Krueger DC, Morgan S, Wiebe D. Current status of clinical 25-hydroxyvitamin D measurement: an assessment of between-laboratory agreement. *Clin Chim Acta* 2010; **411**: 1976–82.

99 Moon HW, Cho JH, Hur M *et al*. Comparison of four current 25-hydroxyvitamin D assays. *Clin Biochem* 2012; **45**: 326–30.

100 Farrell CJ, Martin S, McWhinney B, Straub I, Williams P, Herrmann M. State-of-the-art vitamin D assays: a comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods. *Clin Chem* 2012; **58**: 531–42.

101 Heijboer AC, Blankenstein MA, Kema IP, Buijs MM. Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. *Clin Chem* 2012; **58**: 543–8.

102 Carter GD, Carter R, Jones J, Berry J. How accurate are assays for 25-hydroxyvitamin D? Data from the international vitamin D external quality assessment scheme. *Clin Chem* 2004; **50**: 2195–7.

103 Carter GD, Berry JL, Gunter E *et al*. Proficiency testing of 25-hydroxyvitamin D (25-OHD) assays. *J Steroid Biochem Mol Biol* 2010; **121**: 176–9.

104 Yetley EA, Pfeiffer CM, Schleicher RL *et al.*; Vitamin D Roundtable on the NHANES Monitoring of Serum 25(OH)D. Assay challenges and options for resolving them. NHANES monitoring of serum 25-hydroxyvitamin D: a roundtable summary. *J Nutr* 2010; **140**: 2030S–2045S.

105 Binkley N, Krueger D, Cowgill CS *et al*. Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. *J Clin Endocrinol Metab* 2004; **89**: 3152–7.

106 Nowak M, Harrison SL, Buettner PG *et al*. Vitamin D status of adults from tropical Australia determined using two different laboratory assays: implications for public health messages. *Photochem Photobiol* 2011; **87**: 935–43.

107 Barake M, Daher RT, Salti I *et al*. 25-hydroxyvitamin D assay variations and impact on clinical decision making. *J Clin Endocrinol Metab* 2012; **97**: 835–43.

108 Uitterlinden AG, Fang Y, van Meurs JB, van Leeuwen H, Pols HA. Vitamin D receptor gene polymorphisms in relation to vitamin D related disease states. *J Steroid Biochem Mol Biol* 2004; **89-90**: 187–93

109 Pilz S, Tomaschitz A. Role of vitamin D in arterial hypertension. *Expert Rev Cardiovasc Ther* 2010; **8**: 1599–608

110 Wuerzner G, Burnier M, Waeber B. Should hypertensive patients take vitamin D? *Curr Hypertens Rep* 2012; **14**: 318–23.

111 Swapna N, Vamsi UM, Usha G, Padma T. Risk conferred by FokI polymorphism of vitamin D receptor (VDR) gene for essential hypertension. *Indian J Hum Genet* 2011; **17**: 201–6.

112 Poon AH, Laprise C, Lemire M *et al.* Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy. *Am J Respir Crit Care Med* 2004; **170**: 967–73.

113 Raby BA, Lazarus R, Silverman EK *et al*. Association of vitamin D receptor gene polymorphisms with childhood and adult asthma. *Am J Respir Crit Care Med* 2004; **170**: 1057–65.

114 Vollmert C, Illig T, Altmüller J *et al*. Single nucleotide polymorphism screening and association analysis – exclusion of integrin beta 7 and vitamin D receptor (chromosome 12q) as candidate genes for asthma. *Clin Exp Allergy* 2004; **34**: 1841–50.

115 Wjst M. Variants in the vitamin D receptor gene and asthma. *BMC Genet* 2005; **6**: 2.

116 Butler MW, Burt A, Edwards TL *et al*. Vitamin D receptor gene as a candidate gene for Parkinson disease. *Ann Hum Genet* 2011; **75**: 201–10.

117 Gezen-Ak D, Dursun E, Bilgiç B *et al*. Vitamin D receptor gene haplotype is associated with late-onset Alzheimer's disease. *Tohoku J Exp Med* 2012; **228**: 189–96.

118 Feng M, Li H, Chen SF, Li WF, Zhang FB. Polymorphisms in the vitamin D receptor gene and risk of autoimmune thyroid diseases: a meta-analysis. *Endocrine* 2012; **43**: 318–26.

119 Cooper JD, Smyth DJ, Walker NM *et al*. Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes. *Diabetes* 2011; **60**: 1624–31.

120 Hussein AG, Mohamed RH, Alghobashy AA. Synergism of CYP2R1 and CYP27B1 polymorphisms and susceptibility to type 1 diabetes in Egyptian children. *Cell Immunol* 2012; **279**: 42–5.

121 Ramos-Lopez E, Brück P, Jansen T, Herwig J, Badenhoop K. CYP2R1 (vitamin D 25-hydroxylase) gene is associated with susceptibility to type 1 diabetes and vitamin D levels in Germans. *Diabetes Metab Res Rev* 2007; **23**: 631–6.

122 Ramos-Lopez E, Kahles H, Weber S *et al.* Gestational diabetes mellitus and vitamin D deficiency: genetic contribution of CYP27B1 and CYP2R1 polymorphisms. *Diabetes Obes Metab* 2008; **10**: 683–5.

123 Signorello LB, Shi J, Cai Q *et al*. Common variation in vitamin D pathway genes predicts circulating 25-hydroxyvitamin D Levels among African Americans. *PLoS One* 2011; **6**: e28623.

124 Orton SM, Morris AP, Herrera BM *et al*. Evidence for genetic regulation of vitamin D status in twins with multiple sclerosis. *Am J Clin Nutr* 2008; **88**: 441–7.

125 Bailey R, Cooper JD, Zeitels L *et al*. Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. *Diabetes* 2007; **56**: 2616–21.

126 Kitanaka S, Takeyama K, Murayama A *et al*. Inactivating mutations in the 25-hydroxyvitamin D3 1·-hydroxylase gene in patients with pseudovitamin D-deficiency rickets. *N Engl J Med* 1998; **338**: 653–61.

127 Wang JT, Lin CJ, Burridge SM *et al.* Genetics of vitamin D 1alpha-hydroxylase deficiency in 17 families. *Am J Hum Genet* 1998; **63**: 1694–702.

128 Engelman CD, Fingerlin TE, Langefeld CD *et al*. Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans. *J Clin Endocrinol Metab* 2008; **93**: 3381–8.

129 Berry D, Hyppönen E. Determinants of vitamin D status: focus on genetic variations. *Curr Opin Nephrol Hypertens* 2011; **20**: 331–6.

130 Ahn J, Albanes D, Berndt SI *et al.*; Prostate, Lung, Colorectal and Ovarian Trial Project Team. Vitamin D-related genes, serum vitamin D concentrations and prostate cancer risk. *Carcinogenesis* 2009; **30**: 769–76.

131 Pilz S, Dobnig H, Winklhofer-Roob B *et al.* Low serum levels of 25-hydroxyvitamin D predict fatal cancer in patients referred to coronary angiography. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 1228–33.

132 Thorne J, Campbell MJ. The vitamin D receptor in cancer. *Proc Nutr Soc* 2008; **67**: 115–27.

133 Schwartz Z, Graham EJ, Wang L *et al.* Phospholipase A2 activating protein (PLAA) is required for 1alpha,25(OH)2D3 signaling in growth plate chondrocytes. *J Cell Physiol* 2005; **203**: 54–70

134 Nair-Shalliker V, Clements M, Fenech M, Armstrong BK. Personal sun exposure and serum 25-hydroxy vitamin D concentrations. *Photochem Photobiol* 2012; **89**: 208–14.

135 Feskanich D, Ma J, Fuchs CS *et al*. Plasma vitamin D metabolites and risk of colorectal cancer in women. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 1502–8.

136 Liu G, Wilding G, Staab MJ *et al*. Phase II study of 1alpha-hydroxyvitamin D(2) in the treatment of advanced androgen-independent prostate cancer. *Clin Cancer Res* 2003; **9**: 4077–83.

137 Wactawski-Wende J, Kotchen JM, Anderson GL *et al.*; Women's Health Initiative Investigators. Calcium plus vitamin D supplementation and the risk of colorectal cancer. *N Engl J Med* 2006; **354**: 684–96.

138 Krishnan AV, Feldman D. Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. *Annu Rev Pharmacol Toxicol* 2011; **51**: 311–36.

139 Freedman DM, Chang SC, Falk RT *et al*. Serum levels of vitamin D metabolites and breast cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 889–94.

140 Agic A, Xu H, Altgassen C *et al.* Relative expression of 1,25-dihydroxyvitamin D3 receptor, vitamin D 1a-hydroxylase, vitamin D 24-hydroxylase, and vitamin D 25-hydroxylase in endometriosis and gynecologic cancers. *Reproductive Sciences* 2007; **14**: 486–97.

141 Parikh G, Varadinova M, Suwandhi P *et al*. Vitamin D regulates steroidogenesis and insulin-like growth factor binding protein-1 (IGFBP-1) production in human ovarian cells. *Horm Metab Res* 2010; **42**: 754–7.

142 Henry HL, Norman AW. Vitamin D: metabolism and biological actions. *Annu Rev Nutr* 1984; **4**: 493–520.

143 Tanamura A, Nomura S, Kurauchi O, Furui T, Mizutani S, Tomoda Y. Purification and characterization of 1,25(OH)2D3 receptor from human placenta. *J Obstet Gynaeco* (Tokyo 1995) 1995; **21**: 631–9.

144 Merke J, Hugel U, Ritz E. Nuclear testicular 1,25-dihydroxyvitamin D3 receptors in Sertoli cells and seminiferous tubules of adult rodents. *Biochem Biophys Res Commun* 1985; **127**: 303–9.

145 Johnson JA, Grande JP, Roche PC, Kumar R. Immunohistochemical detection and distribution of the 1,25-dihydroxyvitamin D3 receptor in rat reproductive tissues. *Histochem Cell Biol* 1996; **105**: 7–15.

146 Corbett ST, Hill O,Nangia AK. Vitamin D receptor found in human sperm. *Urology* 2006; **68**: 1345–9.

147 Aquila S, Guido C, Perrotta I, Tripepi S, Nastro A, Ando S. Human sperm anatomy: ultrastructural localization of 1a,25-dihydroxyvitamin D receptor and its possible role in the humanmale gamete. *J Anat* 2008; **213**: 555–64.

148 Blomberg-Jensen M, Nielsen JE *et al*. Vitamin D receptor and vitamin D metabolizing enzymes are expressed in the human male reproductive tract. *Hum Reprod* 2010; **25**: 1303–11.

149 Kwiecinski GG, Petrie GI, DeLuca HF. Vitamin D is necessary for reproductive functions of the male rat. *J Nutr* 1989; **119**: 741–4.

150 Osmundsen BC, Huang HF, Anderson MB, Christakos S, Walters MR. Multiple sites of action of the vitamin D endocrine system: FSH stimulation of testis 1,25-dihydroxyvitamin D3 receptors. *J Steroid Biochem* 1989; **34**: 339–43.

151 Du H, Daftary GS, Lalwani SI, Taylor HS. Direct regulation of

HOXA10 by 1,25-(OH)2D3 in human myelomonocytic cells and human endometrial stromal cells. *Mol Endocrinol* 2005; **19**: 2222–33.
152 Yoshizawa T, Handa Y, Uematsu Y *et al*. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. *Nat Genet* 1997; **16**: 391–6.
153 Kovacs CS, Woodland ML, Fudge NJ, Friel JK. The vitamin D receptor is not required for fetal mineral homeostasis or for the regulation of placental calcium transfer in mice. *Am J Physiol Endocrinol Metab* 2005; **289**: E133–44.
154 Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. *Endocrinology* 2000; **141**: 1317–24.
155 Lerchbaum E, Obermayer-Pietsch B. Vitamin D and fertility: a systematic review. *Eur J Endocrinol* 2012; **166**: 765–78.
156 Ozkan S, Jindal S, Greenseid K *et al*. Replete vitamin D stores predict reproductive success following i*n vitro* fertilization. *Fertil Steril* 2009; **94**: 1314–9.
157 Blomberg-Jensen M, Bjerrum PJ, Jessen TE *et al.* Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa. *Hum Reprod* 2011; **26**: 1307–17.
158 Mathieu C, Gysemans C, Giulietti A, Bouillon R. Vitamin D and diabetes. *Diabetologia* 2005; **48**: 1247–57.
159 Casteels K, Waer M, Bouillon R *et al*. 1,25-dihydroxyvitamin D3 restores sensitivity to cyclophosphamide-induced apoptosis in non-obese diabetic (NOD) mice and protects against diabetes. *Clin Exp Immunol* 1998; **112**: 181–7.
160 Littorin B, Blom P, Schölin A *et al*. Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). *Diabetologia* 2006; **49**: 2847–52.
161 Svoren BM, Volkening LK, Wood JR, Laffel LM. Significant vitamin D deficiency in youth with type 1 diabetes mellitus. *J Pediatr* 2009; **154**: 132–4.
162 Janner M, Ballinari P, Mullis PE, Flück CE. High prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes. *Swiss Med Wkly* 2010; **140**: w13091.
163 Pittas AG, Dawson-Hughes B, Li T *et al.* Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care* 2006; **29**: 650–6.
164 Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM. Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 2005; **28**: 2926–32.
165 Daga RA, Laway BA, Shah ZA, Mir SA, Kotwal SK, Zargar AH. High prevalence of vitamin D deficiency among newly diagnosed youth-onset diabetes mellitus in north India. *Arq Bras Endocrinol Metabol* 2012; **56**: 423–8.
166 Pani MA, Knapp M, Donner H *et al*. Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. *Diabetes* 2000; **49**: 504–7.
167 Chang TJ, Lei HH, Yeh JI *et al.* Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population. *Clin Endocrinol (Oxf)* 2000; **52**: 575–80.
168 Pociot F, McDermott MF. Genetics of type 1 diabetes mellitus. *Genes Immun* 2002; **3**: 235–49.
169 Engelman CD, Meyers KJ, Ziegler JT *et al*. Genome-wide association study of vitamin D concentrations in Hispanic Americans: the IRAS family study. *J Steroid Biochem Mol Biol* 2010; **122**: 186–92.
170 Lasky-Su J, Lange N, Brehm JM *et al.* Genome-wide association analysis of circulating vitamin D levels in children with asthma. *Hum Genet* 2012; **131**: 1495–505.