Review

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D-livering the message: The importance of vitamin D status in chronic liver disease

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Summary

Vitamin D is synthesized predominantly in the liver and functions as an important secosteroid hormone with pleiotropic effects. While its key regulatory role in calcium and bone homeostasis is well established, recently there is increasing recognition that vitamin D also regulates cell proliferation and differentiation, and has immunomodulatory, anti-inflammatory and anti-fibrotic properties. These non-skeletal effects are relevant in the pathogenesis and treatment of many causes of chronic liver disease. Vitamin D deficiency is frequently present in chronic liver disease and may predict non-response to antiviral therapy in chronic hepatitis C. Small studies suggest that vitamin D supplementation improves sustained viral response rates, while 1α-hydroxylase polymorphisms and vitamin D-binding protein are also implicated in therapeutic outcomes. Vitamin D deficiency also closely relates to the severity of non-alcoholic fatty liver disease (NAFLD) and is implicated in the pathogenesis of insulin resistance, a key factor in the development of NAFLD. In preclinical studies, phototherapy and vitamin D supplementation ameliorate NAFLD histopathology, while vitamin D is a powerful anti-fibrotic against thioacetamide liver injury. In liver transplant recipients severe vitamin D deficiency predicts, and vitamin D supplementation prevents, acute cellular rejection. The role of vitamin D in the activation and regulation of both innate and adaptive immune systems may explain its importance in the above liver diseases. Further prospective studies are therefore warranted to investigate the therapeutic impact of vitamin D supplementation in chronic liver disease.

Introduction

Vitamin D is an important secosteroid hormone with pleiotropic effects (Table 1). While its role in the regulation of calcium and bone homeostasis is well established, recently there is increasing recognition that vitamin D has immunomodulatory, anti-inflammatory and anti-fibrotic properties and plays an important role in the regulation of cell proliferation and differentiation. These extraskeletal effects are relevant in the pathogenesis and treatment of many causes of chronic liver disease.

Vitamin D synthesis and metabolism

Vitamin D undergoes a 3-step activation process before it interacts with the vitamin D receptor. The majority of circulating vitamin D is synthesized in the skin as a result of exposure to sunlight. The initial step involves ultraviolet-B radiation (wavelength 290–315 nm) converting the cholesterol metabolite 7-dehydrocholesterol into previtamin D_3 in the lower epidermis, which is rapidly converted to vitamin D_3 in a heat-dependent process. However, excessive sunlight exposure does not cause vitamin D intoxication because excess vitamin D_3 is destroyed by sunlight [1]. Only a small proportion of vitamin D is obtained from dietary sources such as fatty fish, eggs, UV-irradiated mushrooms, supplements, and artificially fortified foods (Table 2). Dietary-derived vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol) are absorbed via a bile-acid dependent process whereby vitamin D is incorporated into micelles in the intestinal lumen, then absorbed by enterocytes and packaged into chylomicrons that are then transported to the venous circulation via lymphatic drainage. Vitamin D from both skin synthesis and dietary sources can either be stored in adipocytes or undergo 25-hydroxylation in the liver. This process is mediated by the 25-hydroxylases, which are cytochrome P450 isoforms that include the important microsomal CYP2R1 and the mitochondrial CYP27A1 enzymes. This produces the main circulating, though biologically inactive, form 25-hydroxyvitamin D [25(OH)D], or calcidiol, which has a long half-life of 2–3 weeks and is therefore used to assess vitamin D status. The vast majority (88%) of serum 25(OH)D is bound to vitamin D-binding protein (DBP), which is also known as Gc or the group-specific component of globulin. DBP is a 58 kDa α-macroglobulin almost exclusively synthesized by the liver and a member of the albumin gene family located on chromosome 4, with high sequence homology to albumin and α-fetoprotein [2]. It is highly polymorphic, having three common isoforms, Gc1F, Gc1S, and Gc2, that display marked racial variation [3], with the Gc1F isoform having the highest affinity for vitamin D metabolites. DBP has anti-inflammatory and immunomodulatory functions independent of its role as the carrier of vitamin D [4,5].

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Table 1. Pleiotropic effects of vitamin D.

Target	Action
Hepatic	
	Inhibits *in vitro* HCV replication in a dose-dependent manner [30-32]
	Supplementation may improve SVR rate in HCV [36-38]
	Vitamin D-binding protein is one of 3 metaproteins associated with SVR in HCV [40]
	Supplementation/phototherapy improves liver histology in preclinical studies of NAFLD [58]
	Supplementation prevents liver fibrosis in preclinical studies [62,63]
	Supplementation decreases risk of acute rejection post-transplantation [125]
Extra-hepatic	
Mortality	Supplementation decreases mortality by 7% [16]
Calcium and bone homeostasis	Enhances Ca and PO_4 absorption from small intestine [6]
	Suppresses PTH secretion [6]
	Induces osteoclast maturation [6]
Pancreas/adipocytes	BMI inversely associated with 25(OH)D level [46]
	Normal vitamin D status associated with 67% lower prevalence of metabolic syndrome [47]
	Activates transcription of insulin gene [50]
	Supplementation improves insulin sensitivity and lowers risk of developing type 2 diabetes [52-54]
Immune system	
Innate	Activates macrophage TLR response to TB infection [95]
	Hastens sputum culture conversion in pulmonary TB in those with tt *Taq*I *VDR* allele [100]
	Downregulates expression of TLR2, TLR4 and TLR9 [107-110]
	Necessary for NK cell development and function [114]
	Enhances NK cell cytotoxicity [115]
	Promotes tolerant DC phenotype by suppressing DC maturation [116]
	Enhances secretion of IL10 and decreases secretion of IL12 from DCs [122]
Adaptive	Activates naïve T cells [117]
	Inhibits proliferation of Th1 lymphocytes [118]
	Shifts balance to a Th2 phenotype [119]
	Increases Treg cells [120,121]
	Inhibits Th17 cell development [121]
	Supplementation decreases risk of developing MS in women [85] and type 1 diabetes in children [86]
Carcinogenesis	Higher 25(OH)D levels associated with lower incidence of colorectal adenoma [135]
	Sunlight exposure associated with reduced risk of NHL [145]

The final step in the synthesis of vitamin D is 1α-hydroxylation that predominantly occurs in the proximal tubule of the kidney but also to a lesser extent in lymphocytes and parathyroid tissue. It is mediated by 1α-hydroxylase (CYP27B1) that produces the active form 1α,25-dihydroxyvitamin D [1α,25$(OH)_2$D] or calcitriol, which is also highly bound to DBP (85%) [2] and has a half-life of only 4 h. 1α,25$(OH)_2$D is the ligand that activates the vitamin D receptor (VDR). This then forms a heterodimer with the retinoid X receptor that acts as a transcription factor that binds to vitamin D response elements in the promoter region of target genes. 1α-hydroxylation is under the influence of factors such as serum phosphate and calcium concentration, parathyroid hormone (PTH), fibroblast growth factor 23 and genetic polymorphisms of *CYP27B1*. 1α,25$(OH)_2$D acts in a negative feedback loop to decrease its own synthesis and increase the expression of 25-hydroxyvitamin D-24-hydoxylase (CYP24A1), which catabolizes 1α,25$(OH)_2$D into calcitroic acid, a biologically inert agent excreted in the bile (Fig. 1).

VDR is expressed in most tissues and cells of the human body, including liver, pancreas, and several immune cells including monocytes, macrophages, T lymphocytes, B lymphocytes, natural killer (NK) cells, and dendritic cells (DC), with expression most abundant on the epithelial cells of the gastrointestinal tract. As a transcription factor activated by 1α,25$(OH)_2$D, VDR directly or indirectly regulates the expression of more than 200 genes that influence cell proliferation, differentiation and apoptosis, as well as immunomodulation and angiogenesis [6]. Studies in VDR null mice highlight the broad physiologic function of vitamin D [7].

Vitamin D deficiency

Vitamin D deficiency is broadly defined as a serum 25(OH)D level <50 nmol/L (<20 ng/ml). Levels between 75 and 125 nmol/L (30–50 ng/ml) are considered optimal as PTH levels rise when 25(OH)D is <75 nmol/L (30 ng/ml); hence, levels between 50

Table 2. Sources of vitamin D.

Vitamin D2 (ergocalciferol):
UV-irradiated mushrooms
Oral supplements
Artificially fortified foods (e.g. milk, cereals, bread, margarine)
Vitamin D3 (cholecalciferol):
Ultraviolet B light (290-315 nm): the major source of vitamin D3
Fatty fish:
Salmon
Mackerel
Tuna
Sardines
Eel
Cod liver oil
Eggs
Oral supplements
Artificially fortified foods (e.g. milk, cereals, bread, margarine)

and 75 nmol/L (20–30 ng/ml) are increasingly recognized to represent vitamin D insufficiency [8–11]. Using these definitions, it is estimated that more than 1 billion people worldwide are either vitamin D deficient or insufficient [12], with the elderly and those with chronic medical illness most at risk. However, even amongst healthy young people, vitamin D deficiency and insufficiency are still common [13–15]. A meta-analysis of 18 randomized-controlled trials involving 57,311 participants shows that subjects randomized to receive vitamin D supplementation, at a mean daily dose of 528 IU, have a statistically significant 7% reduction in all-cause mortality over a mean follow-up duration of 5.7 years [16]. Vitamin D deficiency has a deleterious clinical impact on a number of important medical conditions (Table 3).

Vitamin D and chronic liver disease

The liver is a pivotal organ in the synthesis of vitamin D. It is the site where 25-hydroxylation occurs and where the vast majority of DBP is synthesized. In those with chronic liver disease (CLD) the prevalence of vitamin D insufficiency (<75 nmol/L) is almost universal, with vitamin D deficiency (<50 nmol/L) present in around two-thirds of subjects. Even in the absence of cirrhosis, vitamin D deficiency is present in the majority of subjects. In those with cirrhosis, the prevalence of severe vitamin D deficiency (<25 nmol/L) increases with increasing severity of synthetic liver dysfunction [17,18]. Notably, in those about to undergo liver transplantation, the frequency of 25(OH)D and $1\alpha,25(OH)_2D$ deficiency is 84% and 77%, respectively, with transplantation resulting in a marked increase in 25(OH)D, $1\alpha,25(OH)_2D$, and DBP levels [19].

The high prevalence of vitamin D deficiency in this population occurs regardless of the etiology of liver disease [20,21]. Synthetic liver dysfunction is not entirely responsible, as vitamin D deficiency is still highly prevalent in those with non-cirrhotic liver disease [17]. 25(OH)D levels normalize after oral or parenteral administration of vitamin D in patients with cirrhosis, indicating that 25-hydroxylation is preserved in this patient population [22,23]. Serum DBP levels, which play a critical role in the transport and bioavailability of vitamin D, are moderately decreased in cirrhosis [24,25]. However, as only 5% of DBP binding sites are occupied at any one time with vitamin D metabolites [2], profound liver dysfunction is required for low DBP levels to exert a significant contributing role to vitamin D deficiency in chronic liver disease.

Vitamin D deficiency in CLD is likely to result from a number of mechanisms. In addition to those described above, those patients with a chronic medical illness such as liver disease are more likely to have lower levels of sunlight exposure and/or inadequate dietary intake of vitamin D. Moreover, luminal absorption of dietary sources of vitamin D may be hindered by intestinal edema complicating portal hypertension and/or impaired bile salt dependent micellar incorporation due to cholestasis.

Vitamin D and chronic hepatitis C

Around 170 million people worldwide have chronic hepatitis C (CHC) infection [26], causing a substantial burden of chronic liver disease globally [27]. Vitamin D deficiency is more prevalent in CHC subjects than healthy controls, even in those with minimal liver fibrosis. The majority of subjects with CHC are vitamin D deficient (<50 nmol/L) with 25% having severe deficiency (<25 nmol/L) [28,29]. Current understanding of the mechanisms underlying the high prevalence of vitamin D deficiency in CHC is incomplete.

Nevertheless, recent evidence suggests that vitamin D may impact upon clinical outcomes and treatment response. Fundamental to this are several *in vitro* studies showing that vitamin D inhibits hepatitis C virus (HCV) replication in a dose-dependent manner [30–32]. Moreover, an association between baseline vitamin D status and treatment response to pegylated-interferon (PEG-IFN) and ribavirin (RBV) has recently been established (Fig. 2). Pre-treatment vitamin D deficiency is reportedly an independent predictor of failure to achieve a sustained virologic response (SVR) in HCV genotype 1 (HCV-1), [28,33], and 2/3 infection [29]. However, 25(OH)D level is not associated with SVR in HCV–HIV co-infection [34]. In HCV-1 infection, the rs12979860 C/T polymorphism upstream of the interleukin-28B (*IL28B*) gene on chromosome 19 is the strongest pre-treatment predictor of SVR [35]. Baseline vitamin D status is independent of, but additive to, the *IL28B* genotype in predicting SVR in HCV-1. The highest SVR rate occurs in subjects who have the favorable CC genotype and 25(OH)D levels >50 nmol/L [33].

To date, there is limited data evaluating vitamin D supplementation in CHC treatment. Two small prospective randomized controlled studies from Israel showed that those subjects who received vitamin D_3 supplementation of 2000 IU/day, targeting a 25(OH)D level >80 nmol/L in addition to PEG-IFN/RBV combination therapy, had higher rates of rapid virologic response (RVR; 44% *vs.* 17%, p <0.001), complete early virologic response (cEVR; 94% *vs.* 48%, p <0.001) and SVR (86% *vs.* 42%; OR 2.5, 95% CI 2.0-4.9, p <0.001) in HCV-1 [36] and SVR (95% *vs.* 77%, p <0.001) in HCV-2/3 infection [37] compared to subjects treated with standard therapy. Moreover, recipients of vitamin D_3 supplementation were less likely to be relapsers or non-responders to antiviral therapy, and had improved insulin resistance indices

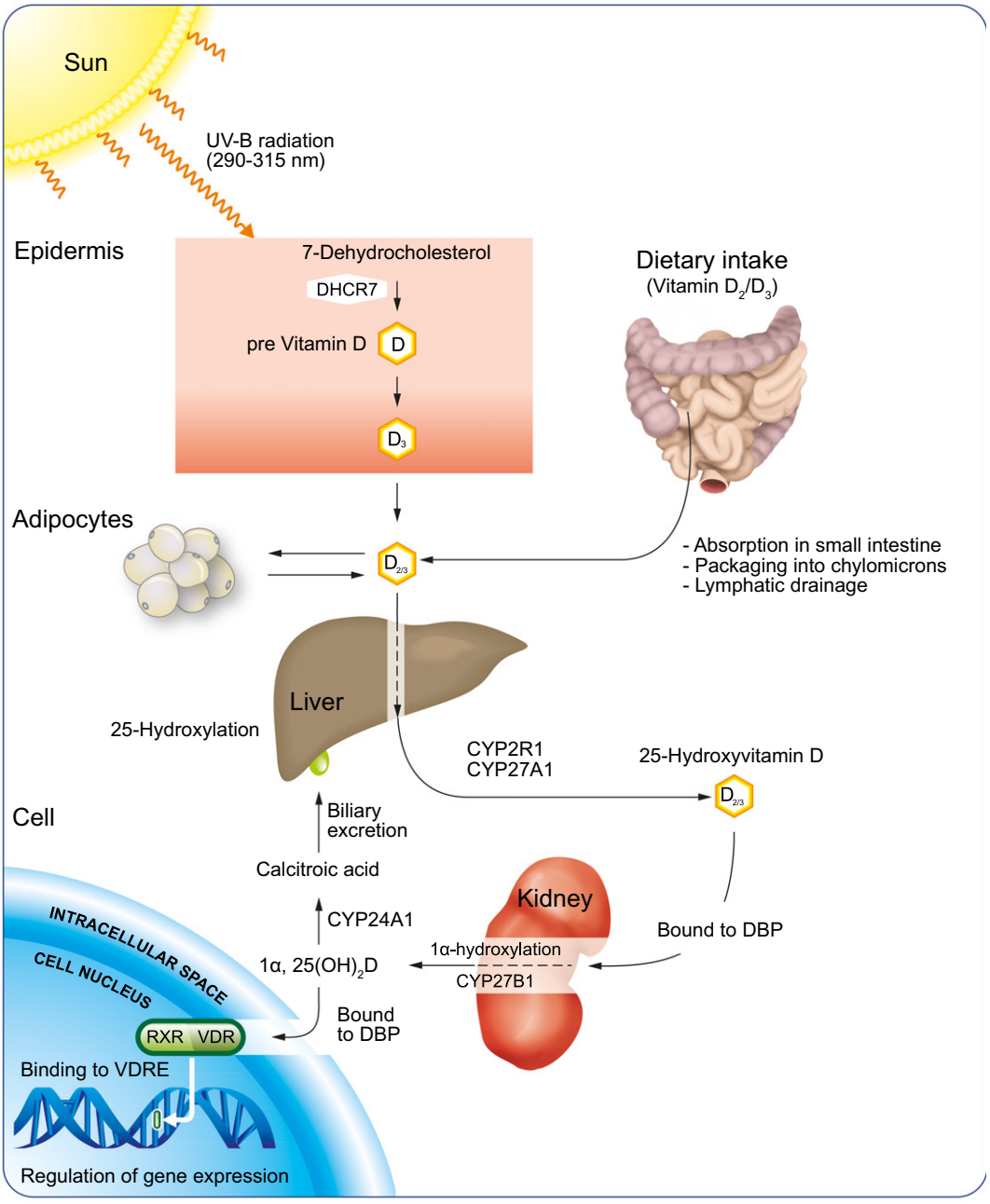

Fig. 1. Vitamin D synthesis.

[36]. Similarly, a small retrospective Italian study showed vitamin D_3 supplementation improved SVR rate in the treatment of recurrent hepatitis C post liver transplantation (53.3% *vs.* 18.5%, $p = 0.02$) [38]. It remains unclear whether these improvements in the clearance of HCV with vitamin D supplementation are the result of an alteration in innate and/or adaptive immune function, or are mediated via improvement in insulin resistance. Large, prospective, placebo-controlled studies are thus required to assess the impact of vitamin D supplementation on viral response in CHC treatment. However, these studies now seem unlikely to occur in the new and rapidly evolving era of direct acting viral therapy.

Vitamin D status also reportedly correlates with liver histology in CHC. Patients with vitamin D deficiency have a higher grade of hepatic necroinflammation [28,33], more advanced fibrosis stage [28,29,34] and may possibly have more rapid fibrosis progression [39]. At a cellular level, vitamin D deficiency is associated with downregulation of the 25-hydroxylase enzyme CYP27A1 in liver tissue. This may have pathogenetic relevance, given the established inverse relationship between CYP27A1 expression and the severity of necroinflammatory activity [28].

The above findings highlight the potential role that proteins and enzymes involved in the synthesis and metabolism of vitamin D may have in liver inflammation and response to anti-viral

Table 3. Clinical impact of vitamin D deficiency.

Target	Action
Hepatic	
HCV	Non-responsiveness to interferon-based therapy [28,29,33]
	More advanced fibrosis stage and higher inflammatory grade [28,29,33]
	May be associated with rapid fibrosis progression [39]
	Downregulation of 25-hydroxylase CYP27A1 in liver [28]
NAFLD	More severe steatosis, inflammation and fibrosis [55,59]
	Increased hepatic expression of TLR2, TLR4 and TLR9 [59], which are implicated in NAFLD pathogenesis [111,112]
Extra-hepatic	
Insulin sensitivity	Associated with presence of insulin resistance [48]
	Predicts future development of insulin resistance and hyperglycaemia [49]
	Impaired pancreatic β-cell function [50,51]
Immune system	Increased risk of *M. tuberculosis* infection [78,92-94]
	Increased population incidence of autoimmune diseases such as MS [84]
Carcinogenesis	Increased risk of colon [136-139], breast [140-142] and prostate cancer [143,144]

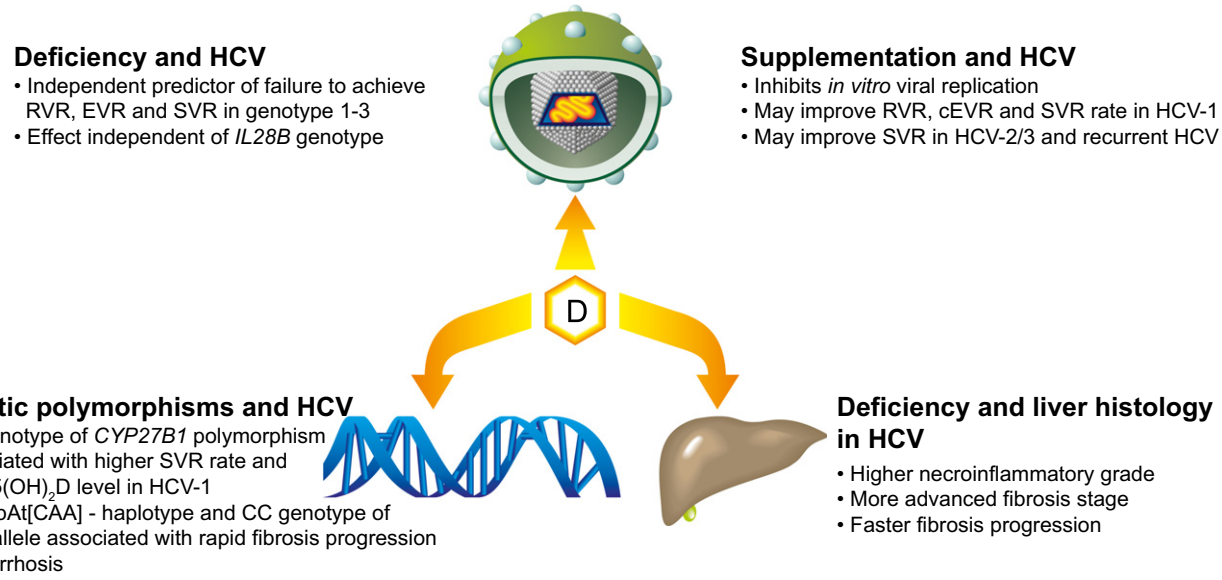

Fig. 2. Vitamin D and hepatitis C.

therapy. Genetic variation in the rs10877012 A/C polymorphism in the promoter region of the 1α-hydroxylase enzyme CYP27B1, but not the rs10735810 *FokI* VDR polymorphism, is associated with SVR in HCV-1 infection. Subjects with the AA genotype have higher SVR rate and 1α,25(OH)$_2$D level than those with the AC or CC genotype [29], suggesting a key role of vitamin D in CHC infection. Moreover, a recently published proteomic study has shown vitamin DBP to be one of three metaproteins associated with SVR [40]. DBP levels are significantly lower in subjects with significant or advanced fibrosis (METAVIR F2-4) compared with those with absent or minimal fibrosis (F0/1) and healthy controls [41,42].

Thus, vitamin D deficiency appears to be common in CHC and may be associated with adverse outcomes such as lower treatment response, more advanced fibrosis stage and increased severity of necroinflammation. It remains, however, uncertain as to whether vitamin D supplementation improves the SVR rate in patients receiving combination anti-viral therapy with PEG-IFN and RBV. Still, the findings of a significant association between the *CYP27B1* rs10877012 A/C polymorphism, higher 1α,25(OH)$_2$D levels, and SVR rate, as well as the association between vitamin D-binding protein and SVR suggest that higher 25(OH)D and 1α,25(OH)$_2$D levels directly improve the virologic response to PEG-IFN and RBV therapy, presumably by impacting on the downstream regulation of vitamin D target gene transcription. DBP determines how much free 25(OH)D substrate is available for 1α-hydroxylase as well as the amount of free 1α,25(OH)$_2$D ligand available to activate the VDR and influences

downstream gene transcription. Hepatic 1α-hydroxylase activity levels therefore represent a major additional factor regulating 1α,25(OH)$_2$D concentration in the liver.

Vitamin D and non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome. It is the most common liver disease in the developed world, with a prevalence of 20–30% [43]. Thirty percent of subjects with NAFLD have histologic evidence of non-alcoholic steatohepatitis (NASH) [44] and are at risk of disease progression and development of cirrhosis. The pathogenesis of NAFLD is yet to be fully elucidated, but insulin resistance (IR) is implicated as the key mechanism leading to hepatic steatosis. Apart from lifestyle modification that results in significant weight loss [45], there is currently no safe, effective therapy for NASH.

Vitamin D levels decrease by 1.3 nmol/L with each 1 kg/m^2 increase in body mass index (BMI) [46]. Normal vitamin D status is associated with a two-thirds lower prevalence of metabolic syndrome compared to those with reduced levels [47]. In non-diabetic Caucasians low vitamin D levels are independently associated with insulin resistance [48] and are a predictor of increased 10-year risk of developing hyperglycemia and insulin resistance [49]. A vitamin D response element is present in the insulin gene promoter region, and 1α,25(OH)$_2$D activates transcription of the insulin gene [50]. Both 1α-hydroxylase and the vitamin D receptor are expressed on pancreatic β cells, with an association between low vitamin D levels and impaired β cell function having been suggested [50,51]. Two randomized placebo-controlled trials have shown that high dose vitamin D supplementation improved insulin sensitivity in non-diabetic South Asians [52,53]. A large prospective cohort study of women demonstrated that those who received vitamin D supplementation had a significantly lower risk of developing type 2 diabetes [54].

Subjects with NAFLD have lower vitamin D levels when compared with controls. Low vitamin D levels are closely associated with histologic severity of steatosis, necroinflammation, and fibrosis in NAFLD, independent of age, gender, BMI, Homeostatic Model Assessment (HOMA)-IR score and presence of metabolic syndrome [55,56]. These findings have been confirmed in children with NAFLD [57].

In a recent study of Lewis rats with diet-induced (choline-deficient and iron-supplemented L-amino acid or CDAA) NASH, phototherapy elevated 25(OH)D, and 1α,25(OH)$_2$D levels while reducing hepatocyte inflammation, fibrosis, and apoptosis when compared to controls. Phototherapy also improved insulin resistance and increased serum adiponectin in association with reduced hepatic expression of the profibrotic transforming growth factor (TGF)-β and α-smooth muscle actin (α-SMA), a marker of hepatic stellate cell activation. In addition, oral vitamin D$_3$ supplementation reportedly improved liver histology in a dose-dependent manner [58]. Furthermore, in a rodent high fat diet model of NAFLD, vitamin D deficiency exacerbated histologic features of NAFLD, increased insulin resistance, and upregulated liver tissue expression of genes involved in hepatic inflammation and oxidative stress [59]. Given the above findings, prospective studies that assess the impact of vitamin D supplementation on the histologic features of NASH are warranted as a priority, given the lack of an effective therapy for this condition.

Vitamin D and liver fibrosis

1α,25(OH)$_2$D has anti-fibrotic effects in lung fibroblasts and mesenchymal multipotent cells *in vitro* [60,61], as well as anti-proliferative and anti-fibrotic effects in both *in vitro* and *in vivo* rat models of liver fibrosis. VDR is expressed by hepatic stellate cells (HSC) and this expression is upregulated by 1α,25(OH)$_2$D. In addition, 1α,25(OH)$_2$D suppresses HSC proliferation, and expression of cyclin D1, tissue inhibitor of metalloproteinase 1 and collagen Iα1 *in vitro*. *In vivo*, 1α,25(OH)$_2$D decreases α-SMA expression and collagen levels, and prevents the development of cirrhosis by thioacetamide (TAA) [62,63]. A vitamin D level >50 nmol/L may be associated with a decreased frequency of rapid fibrosis progression in CHC [39]. However, the clinical importance of vitamin D as an anti-fibrotic agent remains to be determined.

Vitamin D receptor polymorphisms and liver disease

The vitamin D receptor (VDR) gene is located on chromosome 12. It encodes a 48 kDa soluble protein that is a member of the nuclear receptor family of ligand-activated transcription factors. Common single nucleotide polymorphisms (SNP) of the VDR gene include *FokI* (rs10735810), *BsmI* (rs1544410), *ApaI* (rs7975232), and *TaqI* (rs731236). There is a marked racial variation in the allele frequency of these VDR polymorphisms [64], but their influence on VDR function and signaling is unknown [65]. The *BsmI*, *ApaI*, and *TaqI* SNPs are all in the 3′ region of the VDR gene and are in linkage disequilibrium with each other [66].

In CHC infection, the bAt [CCA]-haplotype of the *BsmI*, *ApaI*, and *TaqI* alleles, and the CC genotype of the *ApaI* allele are associated with rapid fibrosis progression, cirrhosis and increased intrahepatic expression of the fibrosis marker gene *MMP-9* [39]. In chronic hepatitis B (HBV) infection, the variation in allele frequency of *BsmI*, *ApaI*, and *TaqI* is associated with HBeAg positivity and HBV flare [67]. Variation in *ApaI*, and to a lesser extent *TaqI*, is also associated with a higher HBV viral load and more severe fibrosis and necroinflammation [68]. Variation in the *TaqI* VDR polymorphism is also associated with both chronic HBV infection [69] and occult HBV infection [70], in which there is a low degree of HBV replication present in HBsAg negative subjects.

In hepatocellular carcinoma (HCC), complicating cirrhosis variation in the allele frequency of the *BsmI*, *ApaI*, and *TaqI*, but not *FokI* VDR polymorphisms is associated with HCC development when compared to cirrhotic patients without HCC. This association is most marked in subjects with alcohol-related cirrhosis, where carriage of the *BsmI–ApaI–TaqI* A–T–C and G–T–T haplotypes is independently associated with an increased risk of HCC. Furthermore, there is a significant difference in allele frequency of these VDR polymorphisms in alcohol-related cirrhosis compared to cirrhosis complicating chronic viral hepatitis [66].

Multiple studies have confirmed an association between VDR polymorphisms and autoimmune liver disease in both European and Asian populations. Variation in the allele frequency of the *BsmI* polymorphism is associated with primary biliary cirrhosis [71,72], while variation of the *FokI* polymorphism is associated with autoimmune hepatitis [64,73]. Furthermore, carriage of the VDR *BsmI–TaqI* G–T/G–T diplotype is an independent predictor of acute cellular rejection post-liver transplantation [74]. Similarly, VDR polymorphisms are associated with a variety of other autoimmune and immune-mediated diseases, including type 1

Table 4. Genetic variation in vitamin D and disease.

Target	Action
Hepatic	
	1α-hydroxylase (*CYP27B1*) gene:
	rs10877012 A/C SNP associated with responsiveness to therapy in HCV-1
	AA genotype has higher SVR rate and 1α,25(OH)$_2$D level than AC or CC genotype [29]
	VDR gene polymorphism associations:
	bAt [CCA]-haplotype of *Bsm*I, *Apa*I and *Taq*I alleles, and CC genotype of the *Apa*I allele predicts rapid fibrosis progression, cirrhosis and increased intrahepatic expression of fibrosis marker gene *MMP-9* [39]
	HBV: eAg positivity and flare [67], higher viral load, more severe fibrosis and necroinflammation [68], chronic infection [69] and occult infection [70]
	*Bsm*I-*Apa*I-*Taq*I A-T-C and G-T-T haplotypes associated with HCC in alcohol-related cirrhosis [66]
	*Bsm*I and PBC [71,72]
	*Fok*I and AIH [64,73]
	*Bsm*I-*Taq*I G-T/G-T diplotype predicts acute rejection post-liver transplantation [74]
Extra-hepatic	
	VDR gene polymorphism associations:
	Immune-mediated diseases: type 1 diabetes [75], leprosy [76], Crohn's disease [77], TB [69,78,100], psoriasis [79], MS [80] and Graves' disease [81]
	Malignancies: melanoma [148] and cancer of the colon [146], ovary [147], breast, prostate and kidney [148]
	DBP gene associations:
	Gc1F, Gc1S and Gc2 isoforms of DBP have differing affinities for vitamin D [133,134] and result in variable responses to vitamin D supplementation [132,133]
	Vitamin D dependent antimicrobial response of monocytes varies with DBP genotype [96]
	Gc2 isoform associated with lower 25(OH)D level [97,98], reduced macrophage function [4] and increased susceptibility to active TB in the presence of severe vitamin D deficiency [99]

diabetes [75], leprosy [76], Crohn's disease [77], tuberculosis [69,78], psoriasis [79], multiple sclerosis [80], and Graves' disease [81] (Table 4).

Vitamin D, the immune system, and the liver

There is an increased incidence and prevalence of autoimmune diseases such as type I diabetes, multiple sclerosis (MS) and Crohn's disease in geographic regions at higher latitude [82,83]. This phenomenon is suggested to be related to lower 25(OH)D levels resulting from decreased ultraviolet sunlight exposure. In support of this hypothesis, the incidence of MS decreases with increasing 25(OH)D levels [84] and vitamin D supplementation decreases the risk of developing both MS in women [85] and type 1 diabetes in children by 80% [86]. In this context, vitamin D has an important role in both the innate and adaptive immune system [87]. Macrophages, T cells, and DCs express both 1α-hydroxylase and vitamin D receptor, and are thus direct targets of 25(OH)D and 1,25(OH)$_2$D [88–90].

Innate immunity

The innate immune response is mediated by pattern-recognition receptors (PRR). Toll-like receptors (TLR) are a family of transmembrane PRRs with broad specificity, expressed on immune cells such as polymorphonuclear cells, monocytes, and macrophages. They interact with pathogen-associated molecular patterns such as viral nucleic acids, and bacterial and fungal products, to trigger an inflammatory (TNF, IL-1β, and IL-6) or antimicrobial response in the host [91]. Several data from studies focusing on the immunology of mycobacterial infection suggest vitamin D and DBP play a significant part in the activation of the innate immune response. The risk of *Mycobacterium tuberculosis* (TB) infection is increased in subjects with vitamin D deficiency, with the greatest risk observed in subjects with the lowest 25(OH)D levels [78,92–94]. At a cellular level, macrophages infected with *M. tuberculosis* initiate a TLR2/1 response that enhances 1α-hydroxylase and VDR expression and induction of the anti-microbial peptide cathelicidin. The anti-microbial activity of macrophages occurs via a vitamin D-dependent process. Addition of 1α,25(OH)$_2$D to *M. tuberculosis*-infected macrophages reduces the number of viable bacilli, while both vitamin D and TLR2/1 are required for cathelicidin production [95]. DBP plays a key role in modulating monocyte responses to 25(OH)D and 1α,25(OH)$_2$D, that varies according to the DBP genotype. Notably, monocytes cultured in serum from *DBP* null mice, and in human serum with the lower affinity Gc1S and Gc2 DBP isoforms, have more potent induction of cathelicidin than monocytes cultured in serum from *DBP*+/− mice or human serum with the high affinity Gc1F isoform [96]. The Gc2 isoform is associated with lower 25(OH)D levels [97,98], and carriers of this allele have an increased susceptibility to active TB in the presence of severe vitamin D deficiency (<20 nmol/L) [99]. The Gc2 isoform is less able to be converted into macrophage activating factor, resulting in reduced macrophage function [4]. These data indicate that vitamin D-dependent antimicrobial responses may be strongly influenced by genetic polymorphisms in DBP, especially in the presence of vitamin D deficiency. Moreover, adjunctive high-dose vitamin D significantly hastens the time to sputum cul-

Table 5. Key future research requirements.

Identification of optimal 25(OH)D level in CLD
Effect of vitamin D supplementation on:
NAFLD
Liver fibrosis and fibrogenesis
Incidence and outcomes of HCC
Outcomes in OLTx recipients
Prospective, randomized, placebo-controlled studies of vitamin D supplementation as an adjunct to HCV anti-viral therapy
Associations and relevance of genetic polymorphisms in *DBP*, *VDR*, 25-hydroxylase and 1-hydroxylase with HBV, HCV, NAFLD and HCC

ture conversion during intensive-phase antimicrobial treatment of pulmonary TB in the subset of patients with the tt genotype of the *TaqI* VDR polymorphism [100]. In addition, variation in allele frequency of the *FokI* and *TaqI* VDR polymorphisms in the presence of vitamin D deficiency is associated with increased risk of TB [69,78].

The role of vitamin D in innate immunity has implications on liver disease. Chronic liver disease is characterized by ongoing increased exposure of the liver via the portal circulation to bacterial products such as lipopolysaccharide (LPS). Contributing factors include increased intestinal mucosal permeability, alcohol ingestion, and small bowel bacterial overgrowth [101–103]. Dietary factors, such as a high-fat diet that predisposes to NAFLD, may also contribute to increased intestinal permeability and result in increased hepatic exposure to LPS [104]. Kupffer cells, the resident macrophages of the liver, represent 80–90% of the macrophages in the body [105], and their innate immune vitamin D-dependent antimicrobial response is also likely to be influenced by the vitamin D status and genetic polymorphisms in DBP. They also express TLR2, TLR4, and TLR9, and are responsive to LPS, the main ligand of TLR4. Hepatocytes, hepatic stellate cells, sinusoidal epithelial cells, biliary epithelial cells, and hepatic DCs also express TLR4 and are responsive to LPS. The interaction of LPS with TLR4 in the liver is crucial during hepatic fibrogenesis [101,106]. Serum vitamin D levels are inversely proportional to TLR2 and TLR4 expression in monocytes, with administration of $1\alpha,25(OH)_2D$ downregulating expression of TLR2, TLR4, and TLR9 [107–110]. Intestinal microbiota play an essential role in hepatic fat accumulation. TLR2, TLR4, and TLR9 are implicated in the pathogenesis of NAFLD, with TLR4 and TLR9 signaling associated with worsening steatosis, inflammation and fibrosis [111,112]. In obese rats, vitamin D deficiency increases hepatic mRNA levels of *TLR2, TLR4*, and *TLR9*, and the endotoxin receptor *CD14*, which is implicated in worsening histologic features of NAFLD [59]. In CHC infection, increasing hepatic necroinflammatory activity correlates with increasing hepatic mRNA expression of *TLR2* and *TLR4*, and hepatic *TNFα* mRNA is also closely correlated with *TLR2* and *TLR4* mRNA expression [113]. Furthermore, the antiviral effect of vitamin D on hepatitis C inoculated HuH7.5 hepatoma cells is mediated by innate immune system activation of the interferon-mediated signaling pathways [30].

NK cells and DCs are both important innate immune effector cells. Studies in VDR knockout mice have shown that expression of VDR is necessary for NK cell development and function [114]. $1\alpha,25(OH)_2D$ enhances NK cell cytotoxicity [115] and suppresses DC maturation, inducing a more tolerant DC phenotype which, at the interface of the innate and adaptive immune systems, promotes T regulatory (Treg, CD4+CD25+) cell activity [116].

Adaptive immunity

Vitamin D is an important modulator of T cell response to pathogens, which is a key component of adaptive immunity. In particular, activation of naïve T cells is a vitamin D-dependent process. In the inactivated state, naïve T cells do not express VDR and express almost no phospholipase C-γ1 (PLCγ1), which is a key molecule required for subsequent classical T cell receptor signaling and T cell activation. Following stimulus exposure, VDR is expressed on T cells through T cell receptor signaling via the alternative mitogen-activated kinase p38 pathway. The VDR complex, activated by binding of $1\alpha,25(OH)_2D$, upregulates transcription of the gene encoding *PLCγ1* and results in a 75-fold increase in PLCγ1 expression, enabling activation of naïve T cells. T cells in patients with lower 25(OH)D and $1\alpha,25(OH)_2D$ levels have a lower proliferation index after stimulation than T cells from patients with normal 25(OH)D and $1\alpha,25(OH)_2D$ levels; this pattern is overcome by exogenous administration of $1\alpha,25(OH)_2D$ [117].

$1\alpha,25(OH)_2D$ also has an anti-proliferative effect on adaptive immunity. It inhibits proliferation of T helper type 1 (Th1) lymphocytes, which produce interferon (IFN)-γ, interleukin (IL)-2, and activate macrophages [118], and shifts the balance to a T helper type 2 (Th2) phenotype with increased production of IL-4, IL-5, and IL-10 [119]. $1\alpha,25(OH)_2D$ increases Treg cells [120,121], enhances DC secretion of IL-10, decreases DC secretion of IL-12, a critical cytokine in Th1 development [122], and inhibits Th17 development via inhibition of IL-6 and IL-23 production [121]. In patients with MS, 25(OH)D, but not $1\alpha,25(OH)_2D$, levels correlate with the ability of Treg cells to suppress the proliferation of activated T responder cells and inversely correlated with Th1/Th2 ratio [123]. *IL-2, IL-10*, and *IL-12* genes in T cells have regions which bind to VDR and $1\alpha,25(OH)_2D$ may directly play a role in the transcription of these cytokines in T cells [124]. The ability of $1\alpha,25(OH)_2D$ to modulate the adaptive immune system may explain the association of vitamin D supplementation and higher 25(OH)D levels with a lower risk of multiple autoimmune diseases.

In orthotopic liver transplant recipients, severe 25(OH)D deficiency (<12.5 nmol/L) and VDR *BsmI–TaqI* G–T/G–T diplotype are independent predictors of moderate-severe acute cellular rejection, whilst vitamin D_3 supplementation decreases the risk of acute rejection by 60% [74,125]. These findings highlight the importance of optimizing the vitamin D status in liver transplant recipients, not only to prevent bone loss, but also to reduce the risk of T cell-mediated acute rejection. A lower Th1/Th2 ratio is an independent predictor of SVR in treatment of HCV-1 [126], which possibly explains why vitamin D supplementation may improve therapeutic outcomes with PEG-IFN plus RBV. The

immune tolerant phenotype promoted by vitamin D may also be of therapeutic benefit in NASH, where activation of both innate and adaptive immunity is implicated in its pathogenesis.

Genome wide association studies of vitamin D

Only about a quarter of vitamin D variability between individuals is explained by factors such as reported dietary intake, latitude and season of measurement [127,128]. Twin and family studies suggest that genetic factors play a significant role in the wide variation of vitamin D levels observed within and between populations [129]. Polymorphisms of the hydroxylases, DBP, and VDR may have a profound influence on serum vitamin D levels and the efficacy of vitamin D as a hormone. Two large genome wide association studies (GWAS), involving patients of European ancestry [130,131], of SNPs and their association with 25(OH)D levels have revealed important information about genetic variation in the enzymes and carrier proteins which are integral to the synthesis and metabolism of vitamin D.

The *NADSYN1*/*DHCR7* locus is closely related to the *de novo* synthesis of vitamin D_3 in the skin from the precursor 7-dehydrocholesterol. There is an association between 25(OH)D levels and several SNPs including rs12785878, rs12800438, rs3794060, rs4945008, and rs4944957 of this locus [124]. SNPs in the 25-hydroxylase *CYP2R1* locus rs10741657, rs2060793, rs12794714, rs10500804, and rs7116978 are also significantly associated with 25(OH)D levels [128,129].

The highly polymorphic vitamin D-binding protein binds the majority of 25(OH)D and 1α,25$(OH)_2$D. DBP is predominantly produced in the liver, but also in kidney, gonads, fat, and neutrophils. SNPs in the *DBP* locus associated with 25(OH)D levels are rs2282679, rs7041, rs3755967, rs17467825, rs2298850, and rs1155563 [124–126]. Response to vitamin D supplementation may vary with differing genotypes of *DBP* [132,133]. In addition to the three common isoforms Gc1F, Gc1S, and Gc2, there are >120 rare variants of DBP. Haplotypes of the SNPs rs4588 and rs7041 in exon 11 of the gene result in the Gc1F, Gc1S, and Gc2 isoforms, which have differing affinities for vitamin D [134]. The *DBP* SNP rs2282679, which has the strongest association with vitamin D levels, lies in intron 12 near the actin subdomain III and may affect DBP binding of 25(OH)D [131].

24-hydroxylase (CYP24A1) is primarily responsible for the inactivation of 25(OH)D and 1α,25$(OH)_2$D. The SNP rs6013897 from this locus is also associated with vitamin D levels [130].

These studies highlight the importance of genetic variation in vitamin D status and may explain in part the varying response seen to vitamin D supplementation. Polymorphisms in four specific loci involved in vitamin D synthesis and metabolism have a significant impact on circulating 25(OH)D levels in patients of European ancestry. Further GWAS that include patients of more diverse racial backgrounds may reveal more genetic associations with the vitamin D status.

Vitamin D and cancer

Vitamin D is also associated with the development of neoplasia. Higher 25(OH)D levels are associated with a lower risk of incident left-sided colorectal adenomas [135]. Multiple meta-analyses, and large prospective and retrospective observational studies have established that vitamin D deficiency is associated with an increased risk of colon [136–139], breast [140–142], and prostate cancer [143,144]. Furthermore, increased sunlight exposure is associated with a reduced risk of non-Hodgkin's lymphoma [145] and VDR polymorphisms are associated with adenocarcinoma in the colon [146], ovary [147], breast, prostate, renal cell carcinoma, and melanoma [148]. With respect to the liver, a variety of VDR polymorphisms are associated with the development of HCC in at-risk patients as detailed above. However, it remains unclear as to whether vitamin D deficiency is associated with an increased risk of HCC.

Conclusions

Vitamin D deficiency is a common problem in chronic liver disease and is closely associated with disease severity. The anti-inflammatory and immune-modulatory properties of vitamin D provide plausible mechanisms by which vitamin D may impact on disease progression and severity, especially in CHC and NASH. However, there are few prospective studies evaluating the effect of vitamin D supplementation in chronic liver disease and these are clearly warranted in the areas of NASH and CHC based on preclinical, and limited retrospective and prospective clinical data. Genetic polymorphisms of the vitamin D receptor and of proteins and enzymes involved in vitamin D synthesis and activation have an association with vitamin D status and severity of liver disease. Further studies are also warranted in this area, to confirm known associations and evaluate other genetic polymorphisms, especially in the vitamin D binding protein, which plays a key role in vitamin D synthesis, activity and bioavailability (Table 5). In the interim period, we recommend vitamin D status to be assessed in all patients with CLD and, if deficiency is present (<50 nmol/L or 20 ng/ml), supplementation with 1000–4000 IU/day of vitamin D3 should be initiated, with the initial dose dependent upon baseline 25(OH)D levels. However, increasing evidence suggests that supplementation should be considered for a 25(OH)D level <75 nmol/L, especially in those considering interferon-based antiviral therapy for CHC. Further prospective studies are required to identify an optimal 25(OH)D level in subjects with CLD.

Key Points

- Extra-skeletal effects of vitamin D include immunomodulatory, anti-inflammatory, and anti-fibrotic properties
- Vitamin D deficiency is frequently present in CLD
- Vitamin D deficiency may independently predict non-response to antiviral therapy in CHC
- Vitamin D supplementation may improve SVR to interferon-based antiviral therapy in CHC genotypes 1, 2 and 3
- Vitamin D deficiency is associated with the histologic severity of NAFLD
- Vitamin D is a plausible therapy for NAFLD because of its insulin-sensitizing, immunomodulatory, anti-inflammatory, and anti-fibrotic properties

References

[1] Holick MF, Garabedian M. Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. In: Favus MJ, editor. Primer on the metabolic bone diseases and disorders of mineral metabolism. Washington, DC: American Society for Bone and Mineral Research; 2006. p. 129–137.

[2] White P, Cooke N. The multifunctional properties and characteristics of vitamin D-binding protein. Trends Endocrinol Metab 2000;11:320–327.

[3] Kamboh MI, Ferrell RE. Ethnic variation in vitamin D-binding protein (GC): a review of isoelectric focusing studies in human populations. Hum Genet 1986;72:281–293.

[4] Yamamoto N, Homma S. Vitamin D3 binding protein (group-specific component) is a precursor for the macrophage-activating signal factor from lysophosphatidylcholine-treated lymphocytes. Proc Natl Acad Sci U S A 1991;88:8539–8543.

[5] Metcalf JP, Thompson AB, Gossman GL, Nelson KJ, Koyama S, Rennard SI, et al. Gc globulin functions as a cochemotaxin in the lower respiratory tract. A potential mechanism for lung neutrophil recruitment in cigarette smokers. Am Rev Respir Dis 1991;143:844–849.

[6] Holick MF, Vitamin D. Deficiency. N Engl J Med 2007;357:266–281.

[7] Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev 2008;29:726–776.

[8] Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006;81:353–373.

[9] Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006;84:18–28.

[10] Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. Lancet 1998;351:805–806.

[11] Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2005;16:713–716.

[12] Bouillon R. Genetic and environmental determinants of vitamin D status. Lancet 2010;376:148–149.

[13] Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S, et al. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 1997;7:439–443.

[14] Tangpricha V, Pearce EN, Chen TC, Holick MF. Vitamin D insufficiency among free-living healthy young adults. Am J Med 2002;112:659–662.

[15] McGrath JJ, Kimlin MG, Saha S, Eyles DW, Parisi AV. Vitamin D insufficiency in south-east Queensland. Med J Aust 2001;174:150–151.

[16] Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Arch Intern Med 2007;167:1730–1737.

[17] Fisher L, Fisher A. Vitamin D and parathyroid hormone in outpatients with noncholestatic chronic liver disease. Clin Gastroenterol Hepatol 2007;5:513–520.

[18] Arteh J, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. Dig Dis Sci 2010;55:2624–2628.

[19] Reese PP, Bloom RD, Feldman HI, Huverserian A, Thomasson A, Shults J, et al. Changes in vitamin D binding protein and vitamin D concentrations associated with liver transplantation. Liver Int 2011 Sep 9. doi: http://dx.doi.org/10.1111/j.1478-3231.2011.02638.x. [Epub ahead of print].

[20] Rode A, Fourlanos S, Nicoll A. Oral vitamin D replacement is effective in chronic liver disease. Gastroenterol Clin Biol 2010;34:618–620.

[21] Malham M, Jørgensen SP, Ott P, Agnholt J, Vilstrup H, Borre M, et al. Vitamin D deficiency in cirrhosis relates to liver dysfunction rather than Aetiology. World J Gastroenterol 2011;17:922–925.

[22] Heaf JG. Hepatic osteodystrophy. Scand J Gastroenterol 1985;20: 1035–1040.

[23] Compston JE. Hepatic osteodystrophy: vitamin D metabolism in patients with liver disease. Gut 1986;27:1073–1090.

[24] Masuda S, Okano T, Osawa K, Shinjo M, Suematsu T, Kobayashi T. Concentrations of vitamin D-binding protein and vitamin D metabolites in plasma of patients with liver cirrhosis. J Nutr Sci Vitaminol (Tokyo) 1989;35:225–234.

[25] Schiødt FV. Gc-globulin in liver disease. Dan Med Bull 2008;55:131–146.

[26] World Health Organization. Fact sheet N°164. http://www.who.int/mediacentre/factsheets/fs164/en/.

[27] Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005;5:558–567.

[28] Petta S, Cammà C, Scazzone C, Tripodo C, Di Marco V, Bono A, et al. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. Hepatology 2010;51:1158–1167.

[29] Lange CM, Bojunga J, Ramos-Lopez E, von Wagner M, Hassler A, Vermehren J, et al. Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy. J Hepatol 2011;54:887–893.

[30] Gal-Tanamy M, Bachmetov L, Ravid A, Koren R, Erman A, Tur-Kaspa R, et al. Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes. Hepatology 2011;54:1570–1579.

[31] Matsumura T, Kato T, Tasaka-Fujita M, Murayama A, Masaki T, Wakita T, et al. 25-hydroxyvitamin D inhibits hepatitis C virus replication and production of the infectious viruses. Hepatology 2011;54:S547A.

[32] Yano M, Ikeda M, Abe K, Dansako H, Ohkoshi S, Aoyagi Y, et al. Comprehensive analysis of the effects of ordinary nutrients on hepatitis C virus RNA replication in cell culture. Antimicrob Agents Chemother 2007;51:2016–2027.

[33] Bitetto D, Fattovich G, Fabris C, Ceriani E, Falleti E, Fornasiere E, et al. Complementary role of vitamin D deficiency and the interleukin-28B rs12979860 C/T polymorphism in predicting antiviral response in chronic hepatitis C. Hepatology 2011;53:1118–1126.

[34] Terrier B, Carrat F, Geri G, Pol S, Piroth L, Halfon P, et al. Low 25-OH vitamin D serum levels correlate with severe fibrosis in HIV–HCV co-infected patients with chronic hepatitis. J Hepatol 2011;55:756–761.

[35] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.

[36] Abu-Mouch S, Fireman Z, Jarchovsky J, Zeina AR, Assy N. Vitamin D supplementation improves sustained virologic response in chronic hepatitis C (genotype 1)-naïve patients. World J Gastroenterol 2011;17:5184–5190.

[37] Nimer A, Mouch A. Vitamin D improves viral response in hepatitis C genotype 2–3 naïve patients. World J Gastroenterol 2012;18:800–805.

[38] Bitetto D, Fabris C, Fornasiere E, Pipan C, Fumolo E, Cussigh A, et al. Vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C. Transpl Int 2011;24:43–50.

[39] Baur K, Mertens JC, Schmitt J, Iwata R, Stieger B, Eloranta JJ, et al. Combined effect of 25-OH vitamin D plasma levels and genetic Vitamin D Receptor (NR 1|1) variants on fibrosis progression rate in HCV patients. Liver Int 2011 Dec 8. doi: http://dx.doi.org/10.1111/j.1478-3231.2011.02674.x. [Epub ahead of print].

[40] Patel K, Lucas JE, Thompson JW, Dubois LG, Tillmann HL, Thompson AJ, et al. High predictive accuracy of an unbiased proteomic profile for sustained virologic response in chronic hepatitis C patients. Hepatology 2011;53:1809–1818.

[41] Ho AS, Cheng CC, Lee SC, Liu ML, Lee JY, Wang WM, et al. Novel biomarkers predict liver fibrosis in hepatitis C patients: alpha 2 macroglobulin, vitamin D binding protein and apolipoprotein A1. J Biomed Sci 2010;17:58.

[42] Gressner OA, Gao C, Siluschek M, Kim P, Gressner AM. Inverse association between serum concentrations of actin-free vitamin D-binding protein and the histopathological extent of fibrogenic liver disease or hepatocellular carcinoma. Eur J Gastroenterol Hepatol 2009;21:990–995.

[43] Lazo M, Hernaez R, Bonekamp S, Kamel IR, Brancati FL, Guallar E, et al. Nonalcoholic fatty liver disease and mortality among US adults: prospective cohort study. BMJ 2011;343:d6891. http://dx.doi.org/10.1136/bmj.d6891.

[44] Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 2011;140:124–131.

[45] Promrat K, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, et al. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. Hepatology 2010;51:121–129.

[46] Stein EM, Strain G, Sinha N, Ortiz D, Pomp A, Dakin G, et al. Vitamin D insufficiency prior to bariatric surgery: risk factors and a pilot treatment study. Clin Endocrinol (Oxf) 2009;71:176–183.

[47] Hyppönen E, Boucher BJ, Berry DJ, Power C. 25-hydroxy vitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. Diabetes 2008;57:298–305.

[48] Liu E, Meigs JB, Pittas AG, McKeown NM, Economos CD, Booth SL, et al. Plasma 25-hydroxyvitamin D is associated with markers of the insulin resistant phenotype in nondiabetic adults. J Nutr 2009;139:329–334.

[49] Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxyvitamin D is predictive of future glycaemic status and insulin resistance. the Medical Research Council Ely Prospective Study 1990–2000. Diabetes 2008;57:2619–2625.

[50] Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab 2007;92:2017–2029.

[51] Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. Am J Clin Nutr 2004;79:820–825.
[52] Nagpal J, Pande JN, Bhartia A. A double-blind, randomized, placebo-controlled trial of the short-term effect of vitamin D3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men. Diabet Med 2009;26:19–27.
[53] von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient – a randomised, placebo-controlled trial. Br J Nutr 2010;103:549–555.
[54] Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, et al. Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care 2006;29:650–656.
[55] Targher G, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, et al. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 2007;17:517–524.
[56] Barchetta I, Angelico F, Del Ben M, Baroni MG, Pozzilli P, Morini S, et al. Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. BMC Med 2011;9:85.
[57] Manco M, Ciampalini P, Nobili V. Low levels of 25-hydroxyvitamin D3 in children with biopsy-proven nonalcoholic fatty liver disease. Hepatology 2010;51:2229.
[58] Nakano T, Cheng YF, Lai CY, Hsu LW, Chang YC, Deng JY, et al. Impact of artificial sunlight therapy on the progress of non-alcoholic fatty liver disease in rats. J Hepatol 2011;55:415–425.
[59] Roth CL, Elfers CT, Figlewicz DP, Melhorn SJ, Morton GJ, Hoofnagle A, et al. Vitamin D deficiency in obese rats exacerbates NAFLD and increases hepatic resistin and toll-like receptor activation. Hepatology 2011 Oct 12. doi: http://dx.doi.org/10.1002/hep.24737. [Epub ahead of print].
[60] Ramirez AM, Wongtrakool C, Welch T, Steinmeyer A, Zügel U, Roman J. Vitamin D inhibition of pro-fibrotic effects of transforming growth factor beta1 in lung fibroblasts and epithelial cells. J Steroid Biochem Mol Biol 2010;118:142–150.
[61] Artaza JN, Norris JC. Vitamin D reduces the expression of collagen and key profibrotic factors by inducing an antifibrotic phenotype in mesenchymal multipotent cells. J Endocrinol 2009;200:207–221.
[62] Abramovitch S, Dahan-Bachar L, Sharvit E, Weisman Y, Tov AB, Brazowski E, et al. Vitamin D inhibits proliferation and profibrotic marker expression in hepatic stellate cells and decreases thioacetamide-induced liver fibrosis in rats. Gut 2011;60:1728–1737.
[63] Reif S, Betov A, Sharvit E, Brazowski E, Weisman Y, Abramovitch S. Vitamin D has a protective antifibrotic effect in rat model of liver fibrosis. Hepatology 2011;54:S747A.
[64] Fan L, Tu X, Zhu Y, Zhou L, Pfeiffer T, Feltens R, et al. Genetic association of vitamin D receptor polymorphisms with autoimmune hepatitis and primary biliary cirrhosis in the Chinese. J Gastroenterol Hepatol 2005;20:249–255.
[65] Adorini L, Vitamin D. Receptor polymorphisms in primary biliary cirrhosis: a functional connection? J Hepatol 2009;50:1071–1073.
[66] Falleti E, Bitetto D, Fabris C, Cussigh A, Fontanini E, Fornasiere E, et al. Vitamin D receptor gene polymorphisms and hepatocellular carcinoma in alcoholic cirrhosis. World J Gastroenterol 2010;16:3016–3024.
[67] Huang YW, Liao YT, Chen W, Chen CL, Hu JT, Liu CJ, et al. Vitamin D receptor gene polymorphisms and distinct clinical phenotypes of hepatitis B carriers in Taiwan. Genes Immun 2010;11:87–93.
[68] Suneetha PV, Sarin SK, Goyal A, Kumar GT, Shukla DK, Hissar S. Association between vitamin D receptor, CCR5, TNF-α and TNF-β gene polymorphisms and HBV infection and severity of liver disease. J Hepatol 2006;44:856–863.
[69] Bellamy R, Ruwende C, Corrah T, McAdam KP, Thursz M, Whittle HC, et al. Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. J Infect Dis 1999;179:721–724.
[70] Arababadi MK, Pourfathollah AA, Jafarzadeh A, Hassanshahi G, Rezvani ME. Association of exon 9 but not intron 8 VDR polymorphisms with occult HBV infection in south-eastern Iranian patients. J Gastroenterol Hepatol 2010;25:90–93.
[71] Halmos B, Szalay F, Cserniczky T, Nemesanszky E, Lakatos P, Barlage S, et al. Association of primary biliary cirrhosis with vitamin D receptor BsmI genotype polymorphism in a Hungarian population. Dig Dis Sci 2000;45:1091–1095.
[72] Tanaka A, Nezu S, Uegaki S, Kikuchi K, Shibuya A, Miyakawa H, et al. Vitamin D receptor polymorphisms are associated with increased susceptibility to primary biliary cirrhosis in Japanese and Italian populations. J Hepatol 2009;50:1202–1209.
[73] Vogel A, Strassburg CP, Manns MP. Genetic association of vitamin D receptor polymorphisms with primary biliary cirrhosis and autoimmune hepatitis. Hepatology 2002;35:126–131.
[74] Bitetto D, Cussigh A, Fornasiere E, Cmet S, Fontanini E, Bignulin E, et al. Recipient vitamin D receptor BsmI–TaqI G–T/G–T diplotype predicts acute cellular rejection in liver transplanted patients. Hepatology 2011;54:S638A.
[75] McDermott MF, Ramachandran A, Ogunkolade BW, Aganna E, Curtis D, Boucher BJ, et al. Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. Diabetologia 1997;40:971–975.
[76] Roy S, Frodsham A, Saha B, Hazra SK, Mascie-Taylor CG, Hill AV. Association of vitamin D receptor genotype with leprosy type. J Infect Dis 1999;179:187–191.
[77] Simmons JD, Mullighan C, Welsh KI, Jewell DP. Vitamin D receptor gene polymorphism: association with Crohn's disease susceptibility. Gut 2000;47:211–214.
[78] Wilkinson RJ, Llewelyn M, Toossi Z, Patel P, Pasvol G, Lalvani A, et al. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. Lancet 2000;355:618–621.
[79] Park BS, Park JS, Lee DY, Youn JI, Kim IG. Vitamin D receptor polymorphism is associated with psoriasis. J Invest Dermatol 1999;112:113–116.
[80] Fukazawa T, Yabe I, Kikuchi S, Sasaki H, Hamada T, Miyasaka K, et al. Association of vitamin D receptor gene polymorphism with multiple sclerosis in Japanese. J Neurol Sci 1999;166:47–52.
[81] Ban Y, Taniyama M, Ban Y. Vitamin D receptor gene polymorphism is associated with Graves' disease in the Japanese population. J Clin Endocrinol Metab 2000;85:4639–4643.
[82] Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr 2004;80:1717S–1720S.
[83] Ponsonby AL, McMichael A, van der Mei I. Ultraviolet radiation and autoimmune disease: insights from epidemiological research. Toxicology 2002;181–182:71–78.
[84] Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 2006;296:2832–2838.
[85] Munger KL, Zhang SM, O'Reilly E, Hernán MA, Olek MJ, Willett WC, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology 2004;62:60–65.
[86] Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 2001;358:1500–1503.
[87] Bikle DD. Vitamin D and the immune system: role in protection against bacterial infection. Curr Opin Nephrol Hypertens 2008;17:348–352.
[88] Adams S, Sharma OP, Gacad MA, Singer FR. Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis. J Clin Invest 1983;72:1856–1860.
[89] Baeke F, Korf H, Overbergh L, van Etten E, Verstuyf A, Gysemans C, et al. Human T lymphocytes are direct targets of 1,25-dihydroxyvitamin D3 in the immune system. J Steroid Biochem Mol Biol 2010;121:221–227.
[90] Sigmundsdottir H, Pan J, Debes GF, Alt C, Habtezion A, Soler D, et al. DCs metabolize sunlight-induced vitamin D3 to 'program' T cell attraction to the epidermal chemokine CCL27. Nat Immunol 2007;8:285–293.
[91] Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature 2007;449:819–826.
[92] Ustianowski A, Shaffer R, Collin S, Wilkinson RJ, Davidson RN. Prevalence and associations of vitamin D deficiency in foreign-born persons with tuberculosis in London. J Infect 2005;50:432–437.
[93] Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. Int J Epidemiol 2008;37:113–119.
[94] Gibney KB, MacGregor L, Leder K, Torresi J, Marshall C, Ebeling PR, et al. Vitamin D deficiency is associated with tuberculosis and latent tuberculosis infection in immigrants from sub-Saharan Africa. Clin Infect Dis 2008;46:443–446.
[95] Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006;311:1770–1773.
[96] Chun RF, Lauridsen AL, Suon L, Zella LA, Pike JW, Modlin RL, et al. Vitamin D-binding protein directs monocyte response to 25-hydroxy- and 1,25-dihydroxyvitamin D. J Clin Endocrinol Metab 2010;95:3368–3376.
[97] Lauridsen AL, Vestergaard P, Hermann AP, Brot C, Heickendorff L, Mosekilde L, et al. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-

binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int 2005;77:15–22.
[98] Abbas S, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D, et al. The Gc2 allele of the vitamin D binding protein is associated with a decreased postmenopausal breast cancer risk, independent of the vitamin D status. Cancer Epidemiol Biomarkers Prev 2008;17:1339–1343.
[99] Martineau AR, Leandro AC, Anderson ST, Newton SM, Wilkinson KA, Nicol MP, et al. Association between Gc genotype and suspectibility to TB is dependent on vitamin D status. Eur Respir J 2010;35: 1106–1112.
[100] Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, et al. High-dose vitamin D_3 during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomized controlled trial. Lancet 2011;377:242–250.
[101] Mencin A, Kluwe J, Schwabe RF. Toll-like receptors as targets in chronic liver disease. Gut 2009;58:704–720.
[102] Fukui H, Brauner B, Bode JC, Bode C. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. J Hepatol 1991;12:162–169.
[103] Parlesak A, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. J Hepatol 2000;32:742–747.
[104] Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761–1772.
[105] Baffy G. Kupffer cells in non-alcoholic fatty liver disease: the emerging view. J Hepatol 2009;51:212–223.
[106] Guo J, Friedman SL. Toll-like receptor 4 signaling in liver injury and hepatic fibrogenesis. Fibrogenesis Tissue Repair 2010;3:21.
[107] Sadeghi K, Wessner B, Laggner U, Ploder M, Tamandl D, Friedl J, et al. Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. Eur J Immunol 2006;36:361–370.
[108] Do JE, Kwon SY, Park S, Lee ES. Effects of vitamin D on expression of Toll-like receptors of monocytes from patients with Behcet's disease. Rheumatology (Oxford) 2008;47:840–848.
[109] Murillo G, Nagpal V, Tiwari N, Benya RV, Mehta RG. Actions of vitamin D are mediated by the TLR4 pathway in inflammation-induced colon cancer. J Steroid Biochem Mol Biol 2010;121:403–407.
[110] Dickie LJ, Church LD, Coulthard LR, Mathews RJ, Emery P, McDermott MF. Vitamin D3 down-regulates intracellular Toll-like receptor 9-induced IL-6 production in human monocytes. Rheumatology (Oxford) 2010;49:1466–1471.
[111] Li L, Chen L, Hu L, Liu Y, Sun HY, Tang J, et al. Nuclear factor high-mobility group box1 mediating the activation of toll-like receptor 4 signaling in hepatocytes in the early stage of nonalcoholic fatty liver disease in mice. Hepatology 2011;54:1620–1630.
[112] Miura K, Seki E, Ohnishi H, Brenner DA. Role of toll-like receptors and their downstream molecules in the development of nonalcoholic fatty liver disease. Gastroenterol Res Pract 2010:362847.
[113] Berzsenyi MD, Roberts SK, Preiss S, Woollard DJ, Beard MR, Skinner NA, et al. Hepatic TLR2 & TLR4 expression correlates with hepatic inflammation and TNF-α in HCV & HCV/HIV infection. J Viral Hepat 2011;18: 852–860.
[114] Yu S, Cantorna MT. The vitamin D receptor is required for iNKT cell development. Proc Natl Acad Sci U S A 2008;105:5207–5212.
[115] Lee JH, Park S, Cheon S, Lee JH, Kim S, Hur DY, et al. 1,25-Dihydroxyvitamin D_3 enhances NK susceptibility of human melanoma cells via Hsp60-mediated FAS expression. Eur J Immunol 2011;40:2937–2946.
[116] Hewison M. Vitamin D and immune function: an overview. Proc Nutr Soc 2012;71:50–61.
[117] von Essen MR, Kongsbak M, Schjerling P, Olgaard K, Odum N, Geisler C. Vitamin D controls T cell antigen receptor signaling and activation of human T cells. Nature Immunol 2010;11:344–350.
[118] Lemire JM, Archer DC, Beck L, Spiegelberg HL. Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. J Nutr 1995;125:1704S–1708S.
[119] Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O'Garra A. 1α,25-dihydroxyvitamin D3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J Immunol 2001;167:4974–4980.
[120] Penna G, Adorini L. 1α,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J Immunol 2000;164: 2405–2411.
[121] Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM. Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile. J Pharmacol Exp Ther 2008;324:23–33.
[122] D'Ambrosio D, Cippitelli M, Cocciolo MG, Mazzeo D, Di Lucia P, Lang R, et al. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-kappaB downregulation in transcriptional repression of the p40 gene. J Clin Invest 1998;101:252–262.
[123] Smolders J, Menheere P, Thewissen M, Peelen E, Tervaert JW, Hupperts R, et al. Regulatory T cell function correlates with serum 25-hydroxyvitamin D, but not with 1,25-dihydroxyvitamin D, parathyroid hormone and calcium levels in patients with relapsing remitting multiple sclerosis. J Steroid Biochem Mol Biol 2010;121:243–246.
[124] Matilainen JM, Räsänen A, Gynther P, Väisänen S. The genes encoding cytokines IL-2, IL-10 and IL-12B are primary $1\alpha,25(OH)_2D_3$ target genes. J Steroid Biochem Mol Biol 2010;121:142–145.
[125] Bitetto D, Fabris C, Falleti E, Fornasiere E, Fumolo E, Fontanini E, et al. Vitamin D and the risk of acute allograft rejection following human liver transplantation. Liver Int 2010;30:417–422.
[126] Shirakawa H, Matsumoto A, Joshita S, Komatsu M, Tanaka N, Umemura T, et al. Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. Hepatology 2008;48:1753–1760.
[127] Livs hits G, Karasik D, Seibel MJ. Statistical genetic analysis of plasma levels of vitamin D: familial study. Ann Hum Genet 1999;63:429–439.
[128] Shea MK, Benjamin EJ, Dupuis J, Massaro JM, Jacques PF, D'Agostino Sr RB, et al. Genetic and non-genetic correlates of vitamins K and D. Eur J Clin Nutr 2009;63:458–464.
[129] Hunter D, De Lange M, Snieder H, MacGregor AJ, Swaminathan R, Thakker RV, et al. Genetic contribution to bone metabolism, calcium excretion, and vitamin D and parathyroid hormone regulation. J Bone Miner Res 2001;16:371–378.
[130] Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2010;376:180–188.
[131] Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Gen 2010;19:2739–2745.
[132] Fu L, Yun F, Oczak M, Wong BY, Vieth R, Cole DE. Common genetic variants of the vitamin D binding protein (DBP) predict differences in response of serum 25-hydroxyvitamin D [25(OH)D] to vitamin D supplementation. Clin Biochem 2009;42:1174–1177.
[133] Chun RF, Peercy BE, Adams JS, Hewison M. Vitamin D binding protein and monocyte response to 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D: analysis by mathematical modeling. PLoS One 2012;7:e30773.
[134] Chishimba L, Thickett DR, Stockley RA, Wood AM. The vitamin D axis in the lung: a key role for vitamin D-binding protein. Thorax 2010;65: z456–462.
[135] Yin L, Grandi N, Raum E, Haug U, Arndt V, Brenner H. Meta-analysis: serum vitamin D and colorectal adenoma risk. Prev Med 2011;53:10–16.
[136] Feskanich D, Ma J, Fuchs CS, Kirkner GJ, Hankinson SE, Hollis BW, et al. Plasma vitamin D metabolites and risk of colorectal cancer in women. Cancer Epidemiol Biomarkers Prev 2004;13:1502–1508.
[137] Gorham ED, Garland CF, Garland FC, Grant WB, Mohr SB, Lipkin M, et al. Optimal vitamin D status for colorectal cancer prevention. Am J Prev Med 2007;32:210–216.
[138] Yin L, Grandi N, Raum E, Haug U, Arndt V, Brenner H. Meta-analysis: longitudinal studies of serum vitamin D and colorectal cancer risk. Aliment Pharmacol Ther 2009;30:113–125.
[139] Giovannucci E. Epidemiology of vitamin D and colorectal cancer: casual or causal link? J Steroid Biochem Mol Biol 2010;121:349–354.
[140] Garland CF, Garland FC, Gorham ED, Lipkin M, Newmark H, Mohr SB, et al. The role of vitamin D in cancer prevention. Am J Public Health 2006;96:252–261.
[141] Abbas S, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D, et al. Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer – results of a large case–control study. Carcinogenesis 2008;29:93–99.
[142] Yin L, Grandi N, Raum E, Haug U, Arndt V, Brenner H. Meta-analysis: serum vitamin D and breast cancer risk. Eur J Cancer 2010;46:2196–2205.
[143] Ahonen MH, Tenkanen L, Teppo L, Hakama M, Tuohimaa P. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). Cancer Causes Control 2000;11:847–852.
[144] Ahn J, Albanes D, Berndt SI, Peters U, Chatterjee N, Freedman ND, et al. Vitamin D-related genes, serum vitamin D concentrations and prostate cancer risk. Carcinogenesis 2009;30:769–776.

[145] Chang ET, Smedby KE, Hjalgrim H, Porwit-MacDonald A, Roos G, Glimelius B, et al. Family history of hematopoietic malignancy and risk of lymphoma. J Natl Cancer Inst 2005;97:1466–1474.
[146] Touvier M, Chan DS, Lau R, Aune D, Vieira R, Greenwood DC, et al. Meta-analyses of vitamin D intake, 25-hydroxyvitamin D status, vitamin D receptor polymorphisms, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 2011;20:1003–1016.
[147] Lurie G, Wilkens LR, Thompson PJ, Carney ME, Palmieri RT, Pharaoh PD, et al. Vitamin D receptor rs2228570 polymorphism and invasive ovarian carcinoma risk: pooled analysis in five studies within the Ovarian Cancer Association Consortium. Int J Cancer 2011;128:936–943.
[148] Köstner K, Denzer N, Müller CS, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. Anticancer Res 2009;29:3511–3536.