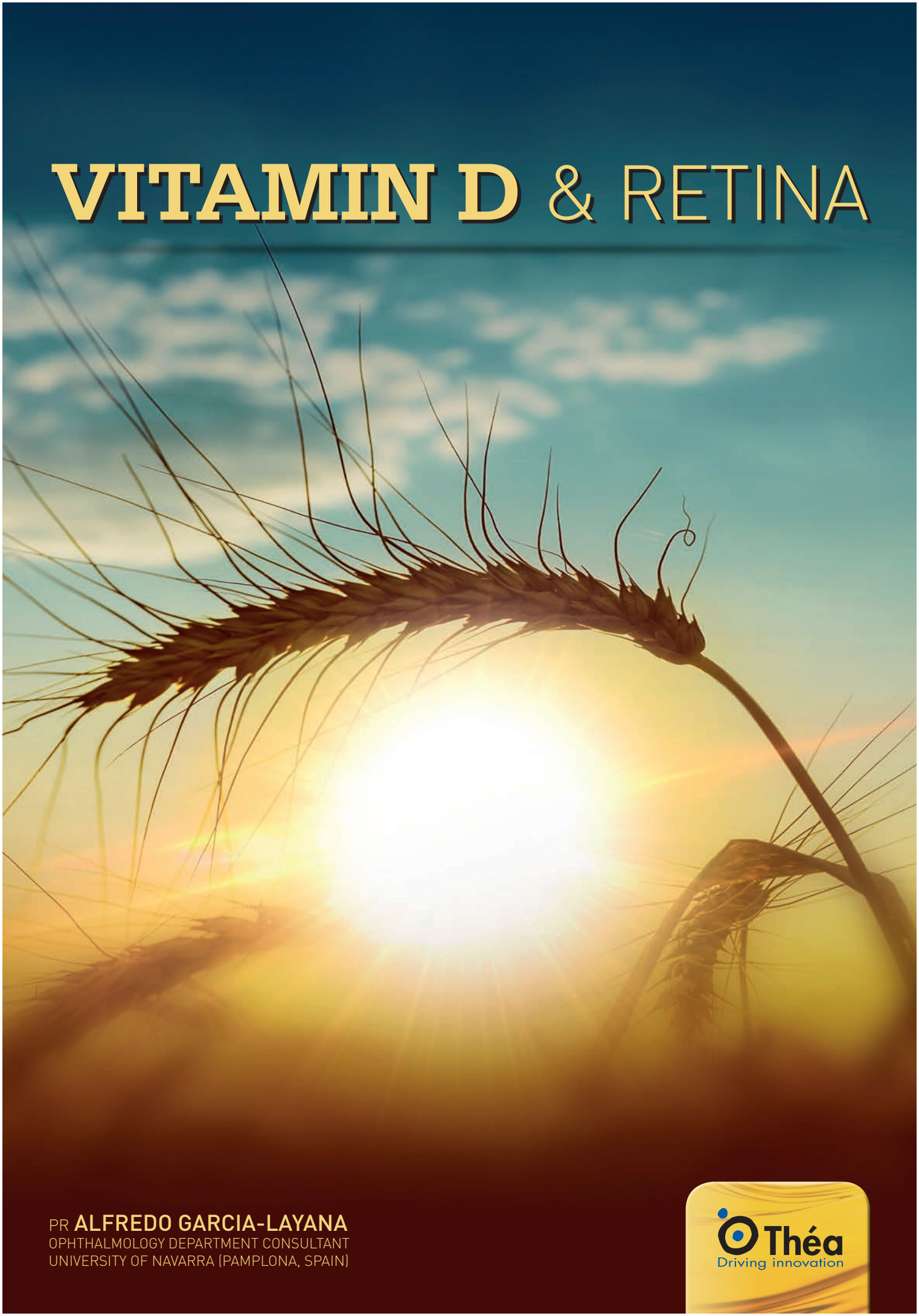
VITAMIN D & RETINA



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SUMMARY

LIST OF ABBREVIATIONS

7-DHC	7-dehydrocholesterol
25(OH)D	25-hydroxilated vitamin D
1,25(OH)$_2$D	1,25-dihydroxilated vitamin D
AD	Alzheimer Disease
AMD	Age-related Macular Degeneration
BMD	Bone Mineral Density
BMI	Body Mass Index
CAREDS	Carotenoids in Age-Related Eye Disease Study
CARMS	Clinical Age-Related Maculopathy Staging
CFH	Complement Factor H
CI	Confidence Interval
CTX	Carboxy-terminal collagen crosslinks
CVD	Cardiovascular Disease
DBP	Vitamin D Binding Protein
DR	Diabetic Retinopathy
DRI	Dietary Reference Intakes
DRIP	Vitamin D Receptor Interacting Protein
EFSA	European Food Safety Authority
FGF23	Fibroblast Growth Factor 23
HEI	Healthy Eating Index
HIF-1	Hypoxia-Inducible Factor-1
HR	Hazard Ratio
HTRA-1	High-temperature requirement factor A1
IL-6	Interleukine-6
IL-8	Interleukine-8
IL-10	Interleukine-10
IL-12	Interleukine-12
IL-17A	Interleukine-17A
IOF	International Osteoporosis Foundation
IOM	Institute Of Medicine
IU	International Unit
MAP	Mitogen-Activated Protein
MCP-1	Monocyte Chemoattractant Protein-1
MMP-9	Matrix Metalloproteinase-9

NHANES III	Third National Health and Nutrition Examination Survey
OCT	Optical Coherence Tomography
OPG	Osteoprotegerin
OR	Odds Ratio
PDGF	Platelet-Derived Growth Factor
PTH	Parathyroid Hormone
RANK	Receptor Activator of Nuclear factor NFκB
RANKL	Receptor Activator of Nuclear factor NFκB Ligand
RDA	Recommended Dietary Allowance
RNFL	Retinal Nerve Fiber Layer
RXR	Retinoid X Receptor
RPE	Retinal Pigmentary Epithelium
SNP	Single Nucleotide Polymorphism
TGFβ	Tumor Growth Factor beta
TLR	Toll-Like Receptor
TNFα	Tumor Necrosis Factor alpha
US	United States
UV	Ultraviolet
VDD	Vitamin D Deficiency
VDR	Vitamin D Receptor
VDRE	Vitamin D responsive element
VEGF	Vascular endothelial growth factor
WHIOS	Women Health Initiative Observational Study

CONVERSION TABLES

25(OH)D concentration		**Vitamin D intake**	
nmol/L	**ng/mL**	**International Unit (IU)**	**μg**
10	4	400	10
30	12	800	20
50	20	1000	25
75	30	1500	37.5

The term vitamin D without a subscript relates to either or both vitamin D2 or vitamin D3 and its metabolites [101]

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EDITORIAL

Age-related macular degeneration (AMD) is the leading cause of visual impairment and blindness in Europe, and has limited treatment options only for its exudative form. Even in those cases where treatment is available, the final visual prognosis is poor. There is not treatment for the geography atrophy that characterizes dry AMD. So, AMD causes a significant proportion of visual loss in the developed world. For those reasons, prevention is mandatory in order to limit the burden of the disease. Currently, little is known about its pathogenesis. Smoke cessation and dietary intake are the most important modifiable risk factors for this condition. The best-validated therapeutic care remain oral antioxidant supplements based on those investigated in the Age-Related Eye Disease Study (AREDS) and the recently completed Age-Related Eye Disease Study 2 (AREDS2). Micronutrition has already become part of the day-to-day management of AMD for a considerable proportion of European ophthalmologists. Although there are obvious differences between general ophthalmologists and retinal specialists and between ophthalmologists from different nations, these differences seem relatively small in the overall context and attributable to different health care environments as well as medical custom and practice. However, European legislation relating to food supplements with vitamins, minerals and other nutritional substances calls for maximum amounts of addition (the Recommended Dietary Allowances or RDA) to protect consumers safety. So, the doses of micronutrients used in the two AREDS studies are not allowed in the EU. In this context, European use of micronutrients in AMD prevention has some unresolved questions and it is difficult to extrapolate AREDS2 results. For instance, there is a controversy about if we can suppress the omega-3 in the European nutritional supplement if we already have a decreased amount of the rest of the components. The control group in AREDS2 received high dose supplements already shown to be effective in the prevention of AMD progression, and moreover, more than 10% added DHA/EPA supplements to their diet. These two factors might be responsible for a "ceiling effect" wherein AREDS2 that could make it more difficult to demonstrate a real effect of omega-3. By contrast, we wonder if we must add other micronutrients to our European supplements in order to achieve the same effect as the AREDS formula. By that reason, the addition of another micronutrients with biological plausibility effects such as resveratrol, vitamin B or vitamin D, may also have a role in the future of AMD prevention.

Vitamin D is a nutrient that has focused the attention of the scientific community, regulatory agencies and the food industry over the last years. Vitamin D deficiency has been described as pandemic throughout the European population, at prevalence rates that are a matter of concern and that require action from a public health perspective. Although there have been some guidelines on sun exposure, the main source of vitamin D, there are doubts about whether a sun exposure would enable vitamin D production without increasing the risk of skin cancer. The increasing sales of vitamin D supplements and the number of fortified food products coming on the market, reemphasize the need for public health strategies for prevention of vitamin D deficiency. Vitamin D has anti-inflammatory, anti-angiogenic, antifibrosis and immune-modulating properties and is hypothesized to protect against the development of AMD. Epidemiologic studies support this hypothesis. In vivo research indicates that both the proteins for the vitamin D receptor and the enzyme, which converts the circulating metabolite of vitamin D to its active form are expressed in the retina. Recent studies have found that deficient vitamin D status is associated with increased odds of AMD. This brochure reviews the current data about vitamin D and its role in health and disease, with special focus in retinal disease and AMD. Current evidence, support that maintenance of adequate vitamin D status, especially in persons at risk for AMD.

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INTRODUCTION

Vitamin D has captured the attention of the scientific and medical communities, regulatory agencies, the food industry, and the public over the past 15 years [1]. This fat-soluble steroid is produced in the skin during sunlight exposition. Therefore, this is not strictly a vitamin (i.e., it is not an essential nutrient), but rather a prohormone that is produced in the skin and transported in blood to target cells and tissues. Vitamin D shows structural analogy with steroid hormones, and works similarly through genomic and non genomic action.

Vitamin D requires sequential enzymatic modification, 25- and 1α-hydroxylation, in the liver and kidney, respectively, to have maximal biological activity as the hormone 1,25(OH)$_2$D. Biologically active vitamin D acts in an endocrine fashion on target organs (i.e. parathyroid, intestine, kidney and bone) to regulate bone, calcium, and phosphate metabolism. Its activity is directly controlled by a specific vitamin D receptor (VDR). The VDR and enzymes involved in the metabolism of vitamin D have been detected in various cells and tissues in the whole body including skin, lung, heart, stomach, pancreas, brain and cells of the immune system. Directly or indirectly, 25(OH)$_2$D controls the expression of more than 200 genes responsible for the regulation of cellular proliferation, differentiation, and apoptosis. Vitamin D may probably acts through autocrine and paracrine pathways.

Thus, beyond its classic bone effect, vitamin D has non classical actions which can be categorised in three general effects: regulation of hormone secretion, regulation of immune function, and regulation of cellular proliferation and differentiation. Retrospective studies have shown inverse relationship between the vitamin D status, and numerous diseases including inflammatory/immune diseases, skin disorders (psoriasis), some cancers, cardiovascular diseases, diabetes, and some brain diseases including depression and dementia. This leads to a vast field of clinical investigations to assess the efficacy and safety of vitamin D supplementation with vitamin D analogues in the treatment of various diseases.

More recently, the VDR has been localised in the eye and it was suggested that vitamin D may influence various ocular diseases, including diabetic retinopathy (DR) and age-related macular degeneration (AMD). Several cross-sectional studies, carried out since a decade, have shown an inverse relationship between DR or AMD and low vitamin D status. Vitamin D may have a protective effect on the development of retinal diseases, mainly through the anti-inflammatory and anti-angiogenetic effects of the biologically active vitamin D. Since a large part of the general population has inadequate vitamin D status according to clinical guidelines, vitamin D and retina is a topic of particular interest. The present brochure exposed all recent aspects of vitamin D in general and in retinal health in particular.

CHAPTER 1

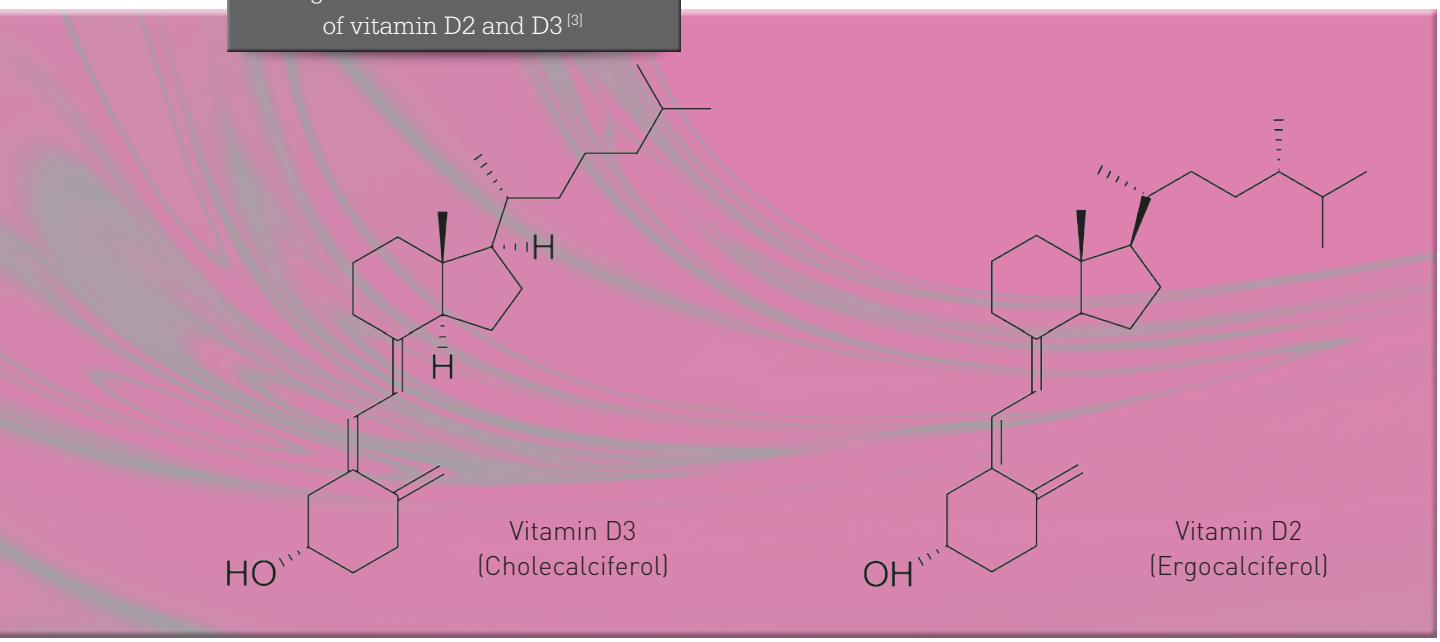
VITAMIN D: METABOLISM AND MODE OF ACTION

1.1 FORMULATION AND NATURAL SOURCE

Vitamin D is a fat-soluble secosteroid. Its molecular structure shows close analogy with classic steroid hormones (e.g., estradiol, cortisol, and aldosterone) with the same root structure (a cyclopentanoperhydrophenanthrene ring), but in vitamin D, the 9,10 carbon-carbon bond of ring B has undergone breakage [2].

There are two main forms of vitamin D which differ by their side chains on the sterol skeleton (Figure 1): vitamin D3 (cholecalciferol) which is produced in the skin by the conversion of 7-dehydrocholesterol (7-DHC) and vitamin D2 (ergocalciferol) which is provided only by foods. Vitamin D without a subscript relates to either or both vitamin D2 or vitamin D3 and its metabolites.

Figure 1: Molecular structure of vitamin D2 and D3 [3]



Skin is the main source of vitamin D. Under the influence of ultraviolet B (UVB) radiation, 7-DHC in the basal strata of the epidermis is converted to previtamin D3, which is immediately converted to vitamin D3 in a heat-dependent process (Figure 2). Radiation in the UVB (290-315 nm) portion of the solar spectrum photolyzes 7-DHC in the skin to previtamin D3, which, in turn, is converted by a thermal process to vitamin D3 [4]. Under normal physiologic circumstances in human, there are ample quantities of 7-DHC available in the *stratum spinosum* and *stratum basale* to photochemically produce vitamin D3 [2].

The formation of previtamin D3 is relatively rapid and reached a maximum within hours after exposition to sunlight radiation [3]. The time needed to produce adequate vitamin D from the skin depends on the strength of the UVB rays and the length of sun exposition time. Skin vitamin D3 formation is thus dependent on the time during the day (more pronounced during mid time), the weather (sunny *versus* cloudy), the seasons (summer *versus* winter), and also on the geographic localization (latitude and altitude).

Endogenous vitamin D3 production also depends on the region of the epidermis exposed, its surface area and its thickness. It was estimated that the exposure of arms and legs for 5 to 30 minutes twice a week was often adequate between the hours of 10 am and 3 pm [3]. Since melanin in the epidermis absorbs one part of the UVB, vitamin D3 production is also dependent on skin pigmentation, dark-skin people having a lower vitamin D status than pale skin [5]. UV protection with sunscreen may also limit the endogenous production of vitamin D [6]. For example, the sunscreen containing para-aminobenzoic acid (sun protection factor 8) may reduce the vitamin D production by 95% [7].

In addition, it is known that the ability to produce vitamin D is diminished with advanced age since ageing results in decreased amount of cutaneous 7-dehydrocholesterol. For instance, vitamin D3 synthesis is reduced approximately by 75% by 70 years of age [3].

Conversely, excessive exposure to sunlight degrades pre-vitamin D3 and vitamin D3 into inactive photoproducts [3], thus preventing from hypervitaminosis D [3].

Table 1: Main factors influencing endogenous synthesis of vitamin D3 [2]

• Quality of UVB radiation (seasons, latitude)
• Quantity (intensity) of UVB
• 7-dehydrocholesterol concentration in the *stratum basale* and *stratum spinosum*
• Melanin concentration in skin

Food is the other source of vitamin D. However most foods with the exception of fatty fish contain little vitamin D unless fortified. Vitamin D2 is produced in plants and fungi (mushrooms). Vitamin D2 and D3 are often used for food fortification in milk, orange juice, yogurt, butter, cheeses, or breakfast cereals (Table 2).

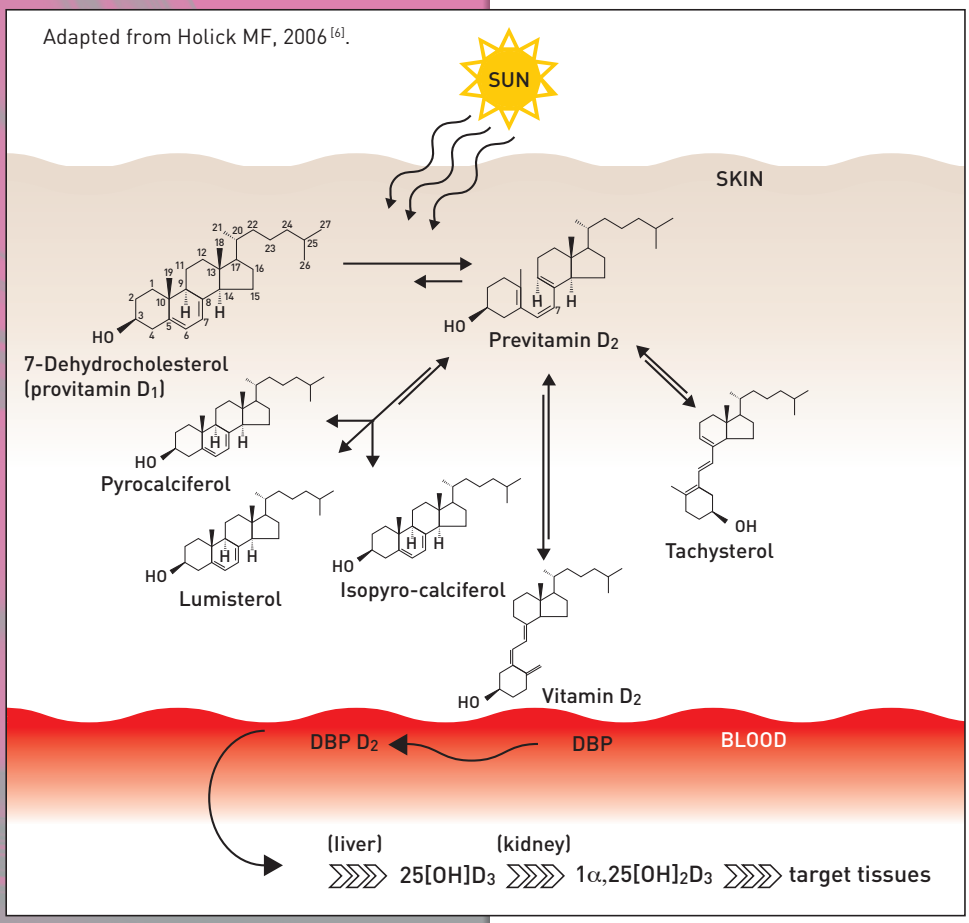
Table 2: Main source of dietary vitamin D [3]

Source	Amount	Vitamin D content*	
Salmon (fresh, wild)	100 g	600 – 1000 IU	i.e. 15 - 25 µg
Salmon (fresh, farmed)	100 g	100 – 250 IU	i.e. 2.5 - 6.25 µg
Salmon (canned)	100 g	300 – 600 IU	i.e. 7.5 - 15 µg
Sardines (canned)	100 g	300 IU	i.e. 7.5 µg
Mackerel (canned)	100 g	250 IU	i.e. 6.25 µg
Tuna (canned)	100 g	230 IU	i.e. 5.75 µg
Cod liver oil	1 teaspoon	400 – 1000 IU	i.e. 10 - 25 µg
Shiitake mushrooms (fresh)	100 g	100 IU	i.e. 2.5 µg
Shiitake mushrooms (sun-dried)	100 g	1600 IU	i.e. 40 µg
Egg yolk	-	20 IU	i.e. 0.5 µg

* 1 international Unit (IU) = 25 ng; Adapted from Holick MF, 2007 [3]

Figure 2: Synthesis of vitamin D3 in skin [2]

Skin exposure to UVB radiations promotes the conversion of 7-dehydrocholesterol (7-DHC) to pre-vitamin D3, which is then converted to vitamin D3 under a heat-mediated process. Excess of pre-vitamin D3 is converted to various inactive metabolites. Vitamin D3 produced in skin binds a specific binding protein (Vitamin D binding protein, DBP), is transported in the blood circulation and converted in the liver into 25-hydroxy vitamin D3 (25(OH)D3), which is then hydroxylated to $1,25(OH)_2$ vitamin D3, the active form of vitamin D, to exert endocrine, paracrine or autocrine action.



1.2 METABOLISM

Once produced in skin, vitamin D is transported in the blood circulation bound to a specific vitamin D binding protein (DBP). Vitamin D from diet or produced by skin is biologically inactive; activation requires enzymatic conversion (hydroxylation) in the liver and kidney. This involves three main enzymatic pathways: 25-hydroxylation, 1-alpha-hydroxylation, and 24-hydroxylation which primarily occurred in liver and kidney (Figure 3) [3].

Two enzymatic hydroxylation reactions in liver and kidney are necessary to convert vitamin D (biologically inactive) to biologically active 1,25 dihydroxy vitamin D (calcitriol).

25-HYDROXYLATION

Vitamin D bound to DBP is transported to the liver and converted by 25-hydroxylation to 25-hydroxyvitamin D (25(OH)D) (Calcidiol). Several enzymes with 25-hydroxylase activity are able to convert vitamin D to 25(OH)D. Most of the 25(OH)D is produced by microsomal CYP2R1 which hydroxylates Vitamin D2 and D3 with comparable kinetics [5, 8]. Another enzyme, the mitochondrial 25-hydroxylase (CYP27A1) is a high capacity, low affinity enzyme and its activity is not generally rate limiting in vitamin D metabolism. CYP27A1 can hydroxylate vitamin D and related compounds at the 24, 25, and 27 positions. While vitamin D3 is preferentially 25-hydroxylated, vitamin D2 is preferentially 24-hydroxylated.

25(OH)D is the most commonly measured vitamin D metabolite to evaluate vitamin D status because of its greater half-life (about 3 weeks) and its serum concentration is up to 1000-fold higher compared with the physiologically active metabolites $1,25(OH)_2D$ produced in kidney (half-life of few hours) [9].

25(OH)D (25-hydroxy vitamin D) is the major circulating form of vitamin D and is used by clinicians to determine Vitamin D status.

1-ALPHA-HYDROXYLATION

25(OH)D is converted to the active metabolite $1,25(OH)_2D$ (Calcitriol) through the action of the enzyme 1-alpha-hydroxylase (CYP27B1) located in the proximal tubule of the kidney, although other tissues have 1-alpha-hydroxylase enzymatic activity.

The synthesis of calcitriol is enhanced by parathyroid hormone (PTH), which levels raise in response to lower levels of serum calcium. Reduced levels of serum phosphate can also increase the production of calcitriol. Its synthesis is also suppressed by the production of fibroblast growth-factor 23 (FGF-23), which is secreted by osteocytes from the bone matrix to balance calcium blood level. In turn, excess of calcitriol inhibits the activity of 1α-hydroxylase (CYP27B1) limiting its own production [15].

24-HYDROXYLATION

CYP24A1 is the only established 24-hydroxylase involved with vitamin D metabolism. In excess, $1,25(OH)_2D$ and 25(OH)D activates CYP24A1 which initiates the degradation of calcitriol by hydroxylation to form calcitroic acid ($1,24,25(OH)_3D$) a biologically inactive product. $1,25(OH)_2D$ is the preferred substrate relative to 25(OH)D, but both are 24-hydroxylated. The primary function of this enzyme is to prevent the accumulation of toxic levels of $1,25(OH)_2D$ and 25(OH)D. CYP24A1 gene expression is upregulated by calcitriol [5, 11].

TRANSPORT AND STORAGE

Vitamin D from food is absorbed by the gastrointestinal tract and transported by the lymphatic system into the venous circulation in chylomicrons [3]. Some vitamin D redistributes to other plasma carriers including lipoprotein, albumin and DBP. Vitamin D2 has a lower affinity for the vitamin binding protein than vitamin D3, resulting in faster clearance from the circulation and lower conversion to active metabolites [8]. Endogenously synthesised vitamin D3 is transported in plasma almost exclusively bound to DBP. This provides a slow hepatic delivery of vitamin D and a sustained increase in plasma 25(OH)D. In contrast, the association of orally administered vitamin D with chylomicrons and lipoproteins allows for receptor-mediated, rapid hepatic delivery of vitamin D, and the reported rapid but less-sustained increases in plasma 25(OH)D [12].

The majority of 25(OH)D and $1,25(OH)_2D$ circulates in large part bound to DBP (85-90%). A lower fraction (10-15%) is bound weakly to albumin, while less than 1% circulates in its free form. Animal studies suggest that DBP serves to protect 25(OH)D from degradation, prolonging its half-life and protecting against vitamin D deficiency. In addition to stabilizing vitamin D concentrations, DBP was shown to slow the action of vitamin D in the intestine and reduce uptake by the liver. It was suggested that only the vitamin D unbound to DPB may be bioavailable and thus active as shown for other steroid hormones (e.g. testosterone) [13, 14].

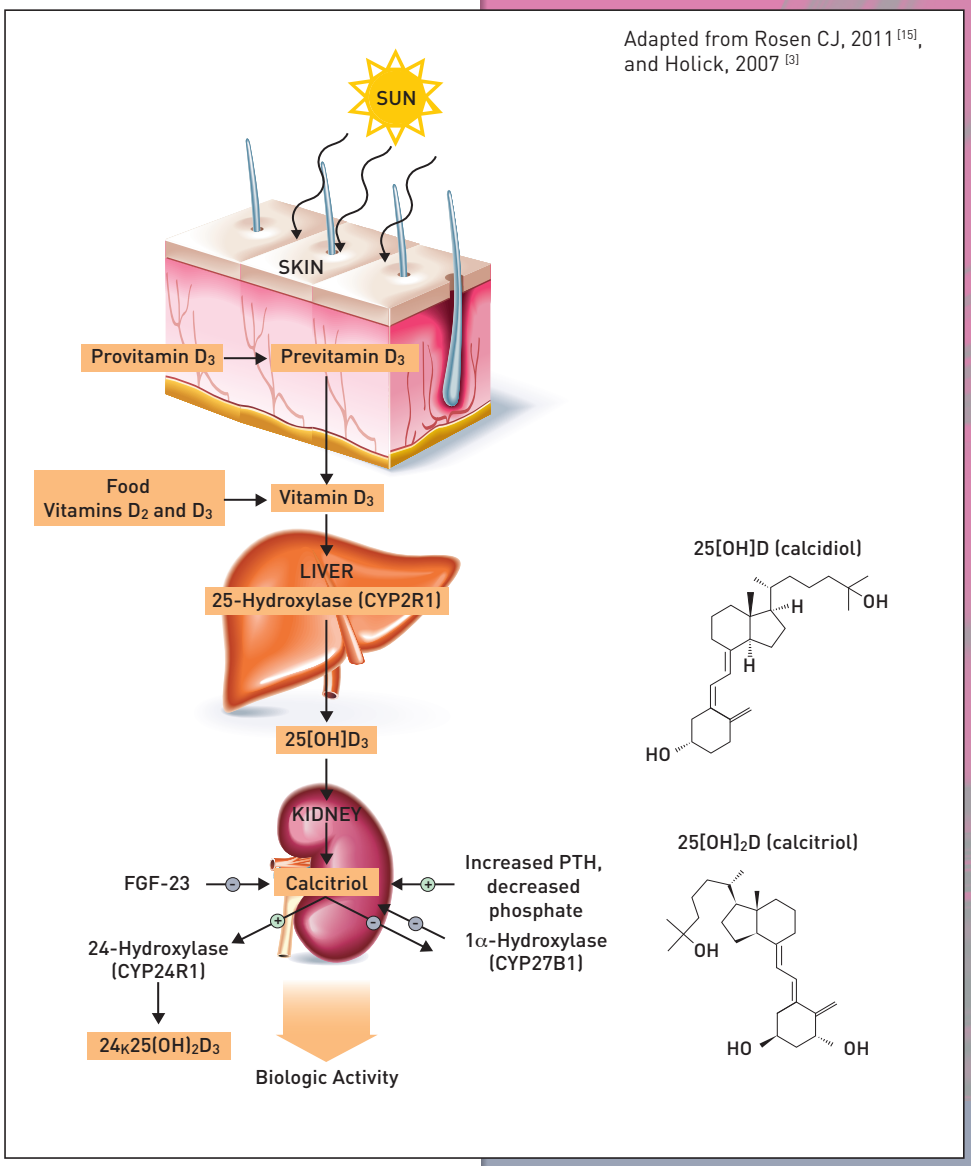


Figure 3: Metabolism of vitamin D

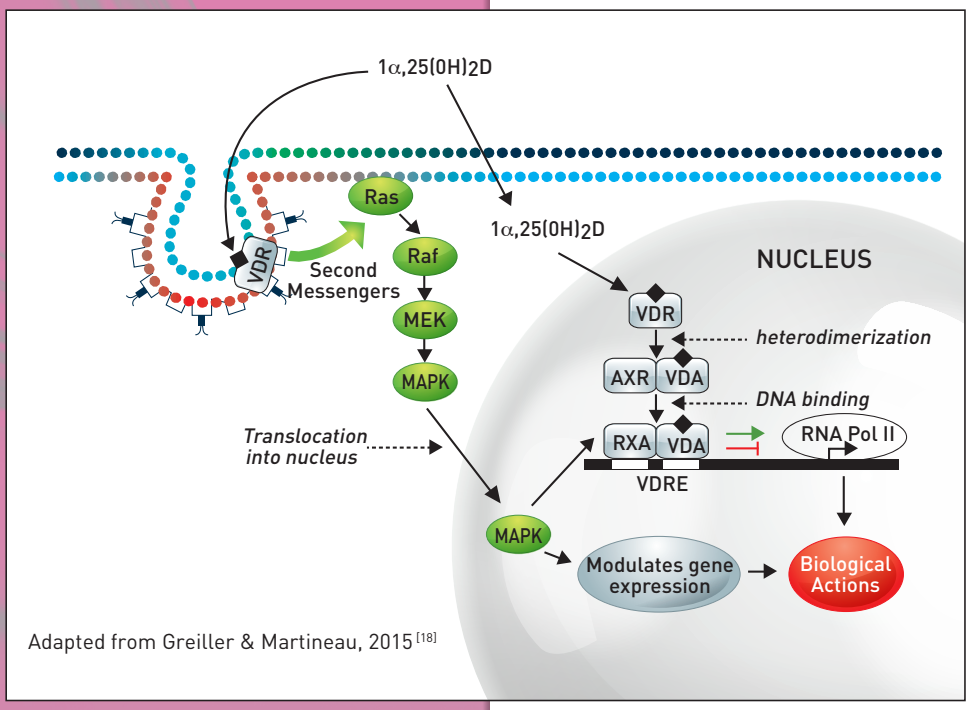
1.3 MECHANISMS OF ACTION

Similarly to steroid hormones, vitamin D functions according to two modes of action: a mechanism mediating gene transcription (genomic action) and a rapid action mediated by the activation of second messengers and the phosphokinases cascade (non genomic action) (Figure 4).

Calcitriol (1.25(OH)$_2$D) is conformationally flexible molecule. It has been suggested that the vitamin D receptor mediating rapid responses uses different ligand (6-s-cis) structural requirements from those utilised by the classic nuclear VDR which signals ligand (6-s-trans) generated genomic responses [16]. Both genomic and non genomic actions may work together, but analogs of different vitamin D isomers that are locked in cis- or trans- conformations have been developed to preferentially elicit rapid non-genomic and/or genomic responses [17].

Figure 4: Genomic and non genomic actions of vitamin D

Genomic action: The $1,25(OH)_2D$ binds to nuclear or membrane vitamin D receptors (VDRs). Nuclear VDR ligation results in heterodimerisation with retinoid X receptor (RXR) and binding to vitamin D responsive elements (VDRE) in promoter regions of responsive genes. Components of the RNA polymerase II complex are then recruited for induction of gene transcription, or transcription is repressed.
Non genomic action: Membrane-associated VDR ligation results in the activation of second messenger systems, with one effect being the initiation of Ras/MAPK signal transduction. Nuclear MAPK modulates gene expression.



Adapted from Greiller & Martineau, 2015 [18]

GENOMIC ACTION

The cellular response of vitamin D is mediated by a complex genomic mechanism involving binding of calcitriol to a specific receptor (VDR) and its RXR coreceptor, and a number of VDR-cofactors leading to cell specific programming of transcriptional responses [11].

The primary molecular action of $1,25(OH)_2D$ is to initiate gene transcription by binding with high affinity to a specific vitamin D receptor (VDR). This receptor is a member of the steroid hormone receptor superfamily of ligand-activated transcription factors.

The sequential steps in gene expression regulated by VDR included (Figure 4):

1) The conformation change of the VDR following interaction with $1,25(OH)_2D$,
2) Formation of the VDR-Retinoid W receptor (RXR) heterodimer,
3) Stabilization of the VDR-RXR heterodimers on responsive element (VDRE),
4) Recruitment of VDR-cofactors including,
5) Transcriptional and translational activity.

These transcriptional coactivators differ in their tissue distribution, providing for substantial tissue specificity in the actions of $1,25(OH)_2D$ and VDR [19].

NON GENOMIC ACTION

In addition to regulating gene expression, $1,25(OH)_2D$ has a number of non genomic actions including the ability to stimulate calcium transport across the plasma membrane. It is now recognised that $1,25(OH)_2D$ can elicit rapid action (within minutes) that are not mediated through transcriptional levels by the VDR (which required several hours or days) [16]. Similar to other steroid hormones, $1,25(OH)_2D$ has been shown to regulate calcium and chloride channel activity, protein kinase C activation and distribution, and phospholipase C activity in a number of cells including osteoblasts, liver, muscle, and intestine [8]. According to cells, the non-genomic response may activate different second messengers system and cytosolic kinases which account for the tissue and cell-specificity of vitamin D biological effects [17].

CHAPTER 2

VITAMIN D AND BONE

The small intestine, kidneys, and bones are the primary organs and tissues responsive to vitamin D and they are involved in mineral metabolism that affects skeletal health [6]. Calcitriol ($25(OH)_2D$) is primarily a calcemic hormone through its actions to stimulate intestinal calcium absorption, bone calcium resorption, and renal calcium reabsorption. Calcitriol also enhances intestinal phosphate absorption. In addition, calcitriol promotes bone formation directly by regulating gene transcription in osteoclast and indirectly by regulating the action of PTH and other hormones (Figure 5).

Table 3: Main function of vitamin D in calcium and phosphate homeostasis

• Stimulation of intestinal calcium and phosphate absorption
• Bone calcium and phosphate resorption
• Renal calcium and phosphate reabsorption

2.1 ACTION ON CALCIUM AND PHOSPHATE ABSORPTION

Calcitriol is the most potent agent with respect to stimulation of intestine calcium and phosphate transport [20].

Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus is absorbed. The interaction of calcitriol with the VDR increases the efficiency of intestinal calcium absorption to 30 to 40% and phosphorus absorption to approximately 80% [3].

The interaction with the VDR receptor and binding to specific VDRE in intestinal enterocytes induce the expression of several key proteins in calcium and phosphate transport [8, 16], including:

- a calcium channel (TRPV6) located at the brush border membrane,
- a 28-kDa calcium-binding protein, called calbindin, which facilitates the transport of calcium in the cytosol,
- a calcium-ATPase, which allows efflux of calcium in the blood stream.

In addition, calcitriol induces rapid transport of calcium (so called transcaltachia) at intestinal level via a non-genomic action [16]. Calcitriol binding to the membrane VDR on enterocytes induces calcium internalization via the endocytic vesicles of the brush-border membrane. The vesicles fuse with lysosomes and circulate along the microtubules to the basolateral membrane of the enterocytes, where the calcium is released by exocytosis through fusion of the lysosomes and the enterocytic membrane.

Similarly, the phosphate intestinal absorption is mediated by a sodium-phosphate transporter (NPt2b) which expression is increased by calcitriol [21].

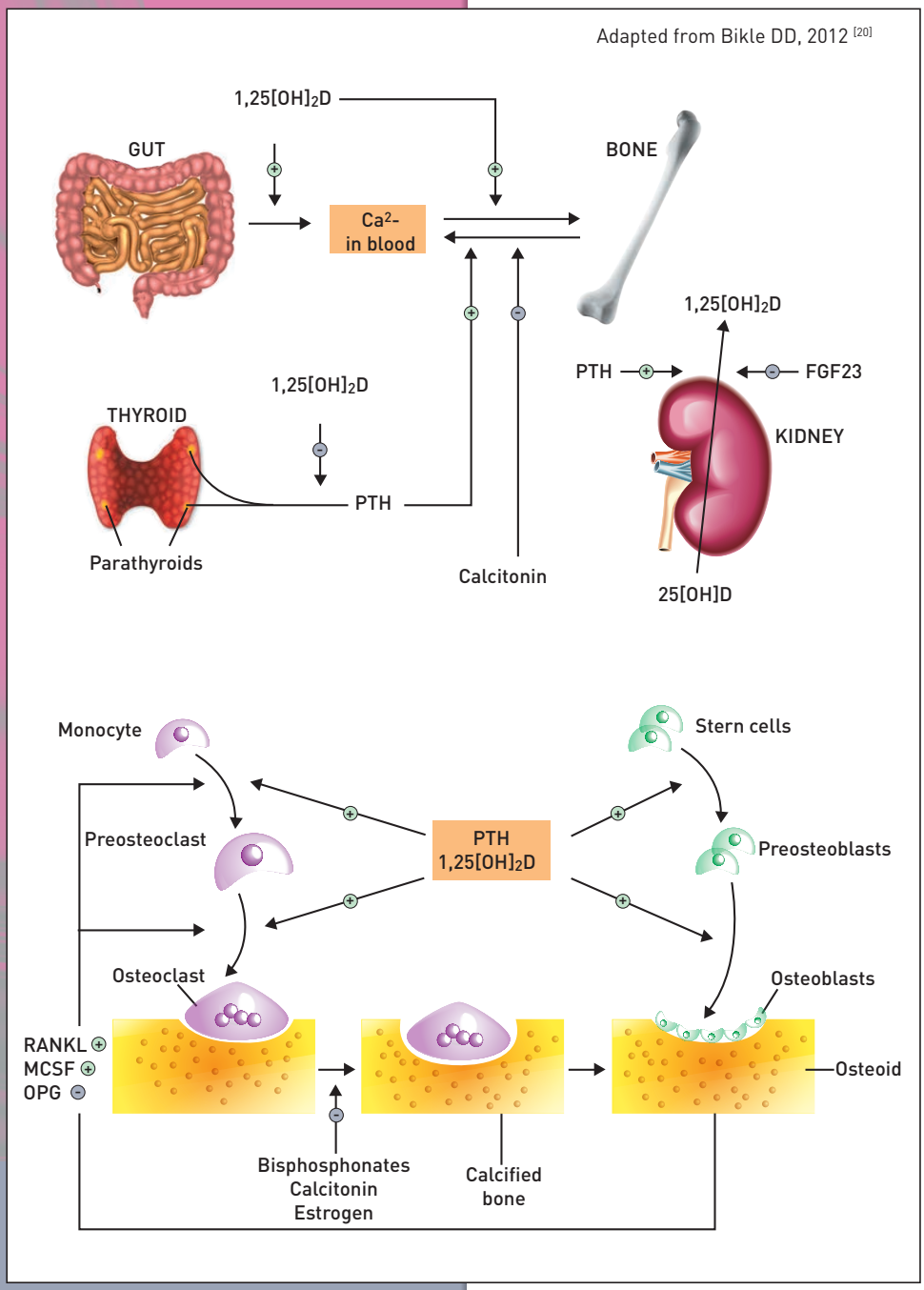
Table 4: Function of vitamin D in calcium intestinal absorption

• Activation of TRPV6 and cellular calcium transport in the intestinal microvilli
• Binding to calmodulin and to brush border myosin 1 (BBM1) in microvilli
• Transfer of calcium in calbindin (CaBP)
• Moving of calcium out of the microvilli and transport in the cytoplasm to the basolateral membrane
• Activation of a calcium-ATPase and release in the blood stream

Renal calcium reabsorption: Most of calcium and phosphate filtered by the renal glomerulus are reabsorbed in the proximal tubule. The efficiency of this renal absorption is increased in presence of calcitriol by a mechanism of calcium transport similar to that in intestinal calcium absorption i.e. by the activity of calcium channel and transport proteins which are directly upregulated by calcitriol (TRPV5, calbindin, and the calcium-ATPase) [8].

Figure 5: Regulation of bone mineral homeostasis by $1,25(OH)_2D$

Vitamin D, together with parathyroid hormone (PTH) and Fibroblast-growth factor 23 (FGF23) are the principal hormonal regulator of bone mineral homeostasis. These hormones act in concert to regulate the concentration of serum calcium and phosphorus (Panel A). Vitamin D promotes the absorption of intestinal calcium in blood and bone, and directly regulates the production of PTH by parathyroid glands. PTH increases the calcium level by stimulating the conversion of 25(OH)D to calcitriol in the kidney through CYP27B1 activation. Moreover, PTH enhances the tubular reabsorption of calcium and also decreases phosphate excretion. Conversely, FGF23, which is specifically produced by osteocytes, acts in the kidney to repress the conversion of 25(OH)D to calcitriol, and enhancing is catabolism through CYP27B1 activation. FGF23, in response to high serum phosphate concentration decreases the reabsorption of phosphate and increases its excretion. (Panel B) Calcitriol directly activates bone cells to regulate the expression of a number of genes. Many of these genes encode bone remodeling effectors that are either catabolic or anabolic, and secreted hormones that influence vitamin D and mineral metabolism. PTH also activates osteoblasts, which stimulate the formation of mature osteoclasts.



2.2 ACTION ON BONE REMODELING

Calcitriol activates the expression of genes involved in bone remodeling including anabolic and catabolic products.

ANABOLIC EFFECT OF VITAMIN D

Calcitriol or 1,25(OH)D induces the expression of several genes involved in bone formation [11]. The vitamin D receptor is expressed in osteoblasts (the cells involved in bone formation) which are target cells for calcitriol action. Calcitriol promotes the proliferation and differentiation of osteoblasts and regulates the production of various proteins such as collagen, alkaline phosphatase, and osteocalcin (BGP) which are important in bone formation to get robust, fracture-resistant bones [8]. Calcitriol induces the gene expression of osteopontin (SPP1) which increases osteoblast survival, and triggers ossification of the skeleton, and LRP5 (LDL-receptor related protein 5), a gene product which stimulate osteoblast proliferation [16].

CATABOLIC EFFECT OF VITAMIN D

In addition to its role in promoting bone formation, calcitriol also promotes bone resorption by increasing the number and activity of osteoclasts. The stimulation of osteoclastogenesis by calcitriol is primarily mediated by osteoblasts. Osteoblasts produce a membrane-associated protein known as RANKL (receptor activator of nuclear factor NFκB ligand) that activates RANK on osteoclasts and their hematopoietic precursors. Osteoprotegerin (OPG), the soluble receptor for RANKL that tempers its activity, is simultaneously repressed to amplify the bioeffect of RANKL [16]. The osteoclasts resorb bone via enzymatic degradation of the collagen matrix and secretion of hydrochloric acid, releasing calcium and phosphorus into the extracellular space [6].

2.3 VITAMIN D AND BONE HEALTH

Vitamin D deficiency is a well-known cause of bone mineralization defect as rickets in children and osteomalacia in adults [22, 23].

As described above, the main effect of the active vitamin D metabolite, calcitriol, is to stimulate the intestinal absorption of calcium. As a consequence, vitamin D deficiency may lead to secondary hyperparathyroidism, responsible of increased bone turnover, mineralization defect, and bone loss which in terms may cause osteoporosis, osteomalacia, and fractures. Epidemiological studies showed relationships between vitamin D deficiency and lower bone mineral density (BMD) in adults, low peak bone mass in children, higher bone turnover and increased fracture incidence [22]. Consistently, vitamin D supplementation studies resulting in an improvement of vitamin D status have demonstrated an increase of BMD, a decrease of bone turnover and a decrease of fracture incidence [22]. Vitamin D supplementation in deficient osteoporotic patients was shown to reduce bone turnover, as shown by lower concentrations of serum or urine biomarkers of bone resorption (osteocalcin, CTX, urinary N-telopeptide) in parallel with increased BMD of spine or hip [22].

In conclusion, vitamin D plays a major role:

- **To promote calcium and phosphate absorption**
- **To maintain bone health**
- **To prevent fractures and osteoporosis**

CHAPTER 3

VITAMIN D NON CLASSICAL ACTIONS

Directly or indirectly, calcitriol controls more than 200 genes, including genes responsible for the regulation of cellular proliferation, differentiation, apoptosis and angiogenesis [3].

Beyond cells involved in regulation of calcium homeostasis and bone health, the circulating levels of calcitriol can potentially influence the activity of other tissues and cells expressing the VDR. In addition, a multitude of *in vitro* studies with human and animal cells have shown that most tissues and cells not only express the VDR but also express the same 1α-hydroxylase (CYP27B1) as the kidney [6]. This included the heart, stomach, pancreas, brain, skin, gonads, the retina and various cells of the immune system [6, 24, 25]. Thus vitamin D acts as an endocrine hormone, but also by autocrine or paracrine mechanisms for local gene regulation [6].

Experimental studies show that the VDR regulates a multitude of biologic effects of vitamin D. In most biological system, vitamin D is a potential modulator of cell proliferation, differentiation and apoptosis.

Table 5: Non-classical action of vitamin D

• Regulation of hormone secretion (PTH, FGF23, Insulin)
• Regulation of immune function
• Regulation of cellular proliferation, differentiation and apoptosis

Calcitriol and synthetic analogs are being investigated for the potential treatment of many pathologic conditions, including psoriasis, infections, autoimmune disease (type 1 diabetes mellitus, rheumatoid arthritis, multiple sclerosis, Crohn disease), hypertension, cardiovascular heart disease, and many common cancers [6, 8]. But for many of the potential applications solid data from randomised clinical trials are still lacking despite promising epidemiologic data and animal studies [5].

3.1 VITAMIN D AND IMMUNE/INFLAMMATORY DISEASES

Vitamin D control the immune/inflammatory response by inhibiting macrophages activation, T cell proliferation, and pro-inflammatory mediators production [19, 26, 27].

Interaction of vitamin D metabolism and the immune system is now well established [25]. Recent reports support a role of calcitriol in mediating normal function of both innate and adaptive immune system (Figure 6).

Vitamin D receptors are present in almost all immune cells, including activated T and B lymphocytes and antigen-presenting cells (monocytes/macrophages, and dentritic cells). Immune cells also express vitamin D activating enzymes, allowing local conversion of inactive vitamin D into calcitriol within the immune system [25, 28].

Biological studies indicate that vitamin D signaling modulate many inflammatory responses at several levels. This includes the regulation of the expression of genes which generate pro-inflammatory mediators, such as cyclooxygenases or 5-lipoxygenase products; the interference with transcription factors, such as NFκB, which regulate the expression of inflammatory genes; and the activation of signaling cascades, such as mitogen-activated protein (MAP) kinases which mediate inflammatory response [27]. Calcitriol down-regulates the activation of the TGFβ signaling pathways, regulates various inflammatory cytokines in a cell-specific manner, down-regulates TNFα gene expression in T-cells. Calcitriol may have positive or negative regulation in monocytes depending on the differentiation state. In addition, there is evidence that calcitriol promote a shift of T-cell responses from a TH1 (inflammatory) toward a TH2 reactions (anti-inflammatory) and suppression of TH17 [29]. Consistently, Laird et al. showed significant associations between low vitamin D status (i.e. 25(OH)D < 25 nmol/L) and markers of inflammation (including the ratio of IL-6 to IL-10 (an anti-inflammatory cytokine) within elderly adults [29].

Vitamin D decreases the production of pro inflammatory cytokines (IL-2, IL-6, IL-8, IL-12, IL-17, TNFα and TGFβ) and increase the production of anti inflammatory cytokines (IL-10) [25, 30].

VITAMIN D AND INFECTION

Low vitamin D status is associated with increased risk of acute infections.

The beneficial effects of UV light exposure on tuberculosis are known since many decades, and are consistent with a role of vitamin D to promote the innate response [25]. It was shown that in macrophages, activation of the toll-like receptor (TLR1/2) heterodimer by *mycobacterium tuberculosis* results in the upregulation of VDR and CYP27B1. Calcitriol acts to modulate toll-like receptor (TLR) signaling (SOCS1 stimulation, p38 MAPK inhibition, and NF-κB activation) leading to reduced production of gene expression and protein release of proinflammatory mediators such as TNFα, IL-6, and MCP-1, and consequently decreased recruitment of monocytes/macrophages and overall inflammation within tissue. In addition, calcitriol increases the production of the antimicrobial peptide cathelicidin and the killing of the intracellular *mycobacterium tuberculosis* [25].

Vitamin D deficiency has been shown to be independently associated with increased risk of viral acute respiratory infection in a number of observational studies. A meta-analysis of clinical trials has demonstrated the protective effects of vitamin D supplementation in prevention of ARI [18].

Several clinical studies have associated lower vitamin D status with increased risk and unfavorable outcome of common acute infections (e.g. cold, influenza, viral respiratory infections, ...) and vitamin D supplementation bolsters clinical responses to acute infection [26]. Vitamin D supplementation is considered to have potential therapeutic implications [26, 31].

VITAMIN D AND IMMUNE DISEASES

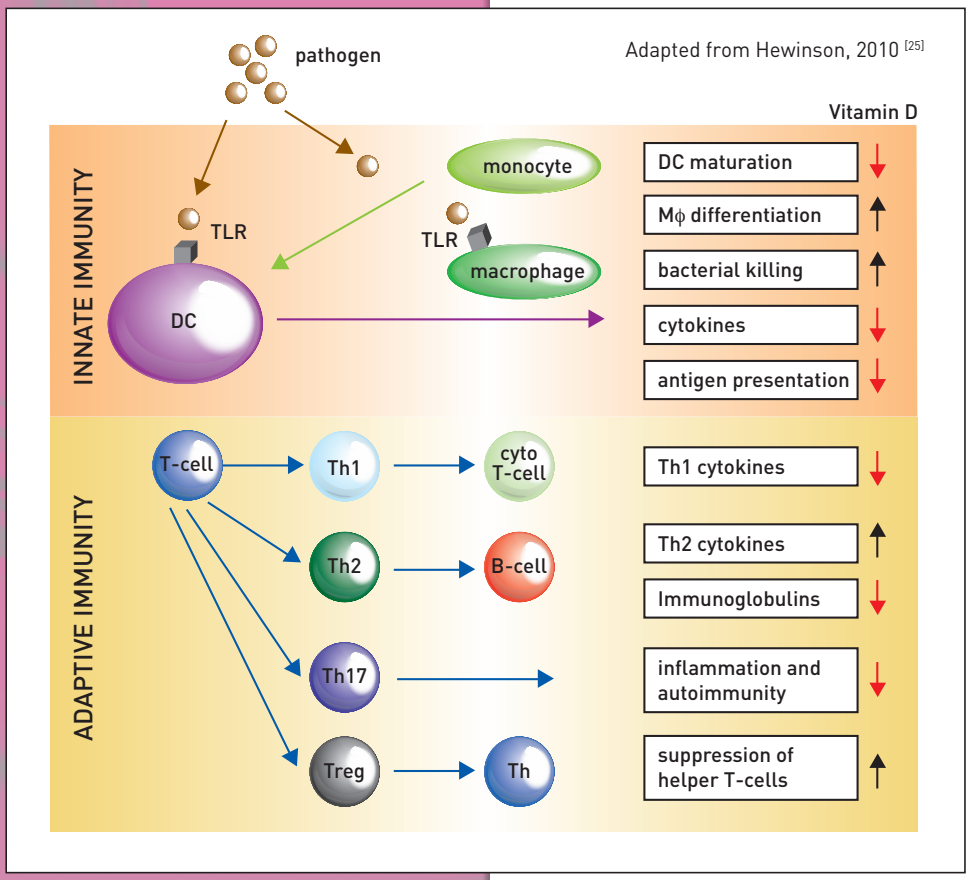
Vitamin D contributes to normal function of the immune system and healthy inflammatory responses [32].

Changes in vitamin D serum levels have been associated with inflammatory diseases such as inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, atherosclerosis, and asthma [27, 33]. Although there is a considerable evidence for an association between vitamin D deficiency and immune/inflammatory diseases, the causality of this association remains to be established. Only limited information is available and existing data are inconsistent regarding the benefit of vitamin D supplementation [27]. Some animal studies demonstrated the benefit of calcitriol or its synthetic analogues in the treatment of autoimmune diseases and as adjuncts to immunosuppressants following transplantation procedures, but as for the treatment of infection, randomised controlled clinical trials are still lacking [5]. Nevertheless, in 2010, an expert panel of the European Food Safety Authority (EFSA) considers that a cause and effect relationship has been established between dietary intake of vitamin D and contribution to normal function of the immune system and healthy inflammatory response [32].

An adequate vitamin D status may be required for optimal immune function, particularly within the older adult population [29].

Figure 6: Effect of calcitriol on the innate and adaptive immune response

Schematic representation of the principal innate and adaptive immune responses to a pathogenic challenge, and the positive or negative regulation of these responses by vitamin D. TLR, toll-like receptor; DC, dendritic cell, MΦ, macrophage; cyto T cell, cytotoxic T-cell; Treg, regulatory T-cell.



3.2 VITAMIN D AND PSORIASIS

Vitamin D reduce the severity of psoriatic lesions by inhibition of cell proliferation [34].

Psoriatic lesions are characterised by increased epidermal proliferation, abnormal keratinocyte differentiation, and infiltration of inflammatory cells [34]. Previous research clearly showed that vitamin D inhibits the proliferation of human keratinocytes and accelerates their differentiation *in vitro*. This has led to the development of topical vitamin D analogs which are currently used in the treatment of psoriasis to reduce the severity and area of psoriatic lesions, with little or no adverse effects [5, 6].

Th17 cytokines are important mediator of psoriatic inflammation and IL-17A is known to play a central role in psoriasis pathogenesis and correlates with disease severity. Recently, it was suggested that vitamin D may inhibit the psoriatic inflammation by blocking IL-17 receptors and NFκB signaling in keratinocytes. In addition, a synthetic derivative of calcitriol (calcipotriol) can inhibit the expression of antimicrobial peptides which are overexpressed in inflammatory skin disease, probably by blocking IL-17 receptors and NFκB signaling in keratinocytes [34].

3.3 VITAMIN D AND CANCER

Vitamin D has antiproliferative and pro-differentiating activity in cancer cells [25].

Serum 25(OH)D levels have been inversely associated with the risk of many types of cancer (including colon carcinoma, breast cancer, prostate cancer...) [19]. It is known that prostate, breast, colon, lung, skin and other organs have the enzymatic machinery (CYP27B) to convert 25(OH)D to $1,25(OH)_2D$, suggesting autocrine or paracrine functions of vitamin D [8]. The mechanisms by which calcitriol can suppress tumor development are numerous and in many cases cell specific. These include inhibition of proliferation by blocking elements of the cell cycle or interference with signaling of growth factors, inducing apoptosis, stimulation of DNA damage repair, prevention of tumor angiogenesis, and inhibition of metastasis [5]. Several *in vitro* studies have shown that breast, colon, and prostate cancer cells, osteosarcomas, and melanomas are responsive to the antiproliferative effects of calcitriol.

However, there are still no studies to support a relationship between total vitamin D intake and cancer risk. For example, a meta-analysis of case-control studies of those with and without colorectal cancer found that, for each 20 ng/mL increase in serum 25(OH)D levels, the odds of colon cancer were reduced by more than 40% (OR, 0.57; 95% CI, 0.43-0.76) [35]. However, in post-menopausal women, the WHI trial failed to demonstrate a significant effect of 400 IU/day vitamin D (plus calcium) supplementation on the risk of colorectal cancer during 7 years of follow-up [36]. Similar observations have been observed for breast and prostate cancer [37]. Thus, further clinical trials with cancer incidence or mortality as a primary outcome need to be conducted to support causality between vitamin D and cancer [28].

3.4 VITAMIN D AND CARDIOVASCULAR DISEASES

Vitamin D deficiency is present in almost all patients with acute myocardial infarction in a multicenter United States cohort [38].

Vitamin D receptors are present in vascular smooth muscle, endothelium, and cardiomyocytes and may have an impact on cardiovascular diseases (CVD) [33]. Vitamin D exerts a variety of favorable effects on endothelial dysfunction, vascular smooth muscle cells proliferation and migration, and calcification, as well as on the inflammatory/immune process of atherosclerosis [39]. Moreover, it exerts beneficial effects against systemic conditions that promote atherosclerosis such as insulin resistance, β-cell dysfunction, dyslipidemia, therefore suggesting a potential therapeutic role [39]. Vitamin D deficiency activates the renin-angiotensin-aldosterone system and can predispose to hypertension and left ventricular hypertrophy [40]. In addition, vitamin D deficiency causes an increase in parathyroid hormone, which increases insulin resistance and is associated with diabetes, hypertension, inflammation, and increased cardiovascular risk. Thus, low vitamin D level is now recognised as a new risk factor for cardiovascular diseases [39].

The majority of observational studies suggested an inverse association between serum 25(OH)D levels and clinical CVD events. Concentrations of 25(OH)D were shown to be inversely associated with CVD mortality among adults with hypertension in the United States. This is consistent with the result of the Framingham Offspring Study and the Health Professionals Follow-up Study, showing an approximately doubled risk for cardiovascular events in vitamin D-deficient subjects [39]. This was confirmed recently in a study involving 10170 women and men from the Danish general population without vitamin D-fortified food, followed for 29 years. Decreasing plasma 25(OH)D levels were associated with increasing risk of ischemic heart disease, myocardial infarction, and early death as a function of seasonally adjusted percentile categories. In the meta-analyses of 18 and 17 studies, risk of ischemic heart disease and early death were increased by 39% and 46% for lowest *versus* highest quartile of 25(OH)D level [41].

Although observational studies suggest that vitamin D deficiency is related to a higher risk for CVD, numerous interventional studies showed conflicting results [39]. Rigorous large-scale, randomised, clinical trials to test the effects of vitamin D (D3 and D2) on atherosclerosis and CVD as the primary outcome are needed to firmly establish the role of vitamin D supplementation on CVD risk and mortality [39].

3.5 VITAMIN D AND DIABETES

The vitamin D status of patients with diabetes should be considered during their regular follow-up, and supplementation should be provided to those at risk of deficiency [42].

Some studies in animal models and humans have suggested that vitamin D may also play a role in the homeostasis of glucose metabolism and the development of type 1 and type 2 diabetes mellitus [33]. Several physiologic mechanisms have been proposed, including the effect of vitamin D on insulin secretion, the direct effect of calcium and vitamin D on insulin action, and the role of this hormone in cytokine regulation [33]. The pancreatic β cell expresses the VDR, and calcitriol was shown to promote insulin secretion and vitamin D insufficiency to be associated with insulin resistance [41b]. In addition, vitamin D deficiency has been associated with microvascular complications in diabetic patients such as diabetic retinopathy [42, 43]. Clinical trials in diabetic or prediabetic patients suggest a benefit from vitamin D administration with respect to improving or preventing the development of frank diabetes, but this needs to be demonstrated in longer and larger randomised clinical trials [5].

3.6 VITAMIN D AND DEMENTIA

In a recent prospective population-based study in USA, the risk of development of dementia was higher in severely 25(OH)D deficient patients (< 25 nmol/L) with an adjusted hazard ratio (HR) of 2.25 (95% CI: 1.23-4.13) for all-cause dementia and 2.22 (95%CI: 1.02-4.83) for incident Alzheimer disease compared to participants without vitamin D deficiency (i.e. 25(OH) D ≥ 50 nmol/L). In deficient patients with 25(OH)D between 25 and 50 nmol/L, the risk for all-dementia and AD were also statistically significantly increased (HR=1.53 [95% CI: 1.06-2.21] and 1.69 [95%CI: 1.06-2.69], respectively). These results support the hypothesis that vitamin D may be neuroprotective [44]. A number of potential mechanisms linking low vitamin D levels with the risk of dementia has been identified [44, 45]. Low vitamin D concentrations may increase the risk of dementia and Alzheimer disease (AD) through both neurodegenerative and vascular mechanisms. Vitamin D receptors are expressed throughout the brain, including areas involved in memory such as the hippocampus and dentate gyrus. In addition, the enzyme that synthesises the active form of vitamin D is produced in several cerebral regions. Calcitriol regulates neurotrophin expression, such as nerve growth factor, neurotrophin 3, and glial-derived neurotrophic factor, and the survival, development, and function of neural cells. *In vitro*, vitamin D stimulates macrophages, which increases the clearance of amyloid plaques. Vitamin D also reduces amyloid-induced cytotoxicity and apoptosis in primary cortical neurons [44, 46].

3.7 VITAMIN D AND DEPRESSION

It has also been suggested that vitamin D deficiency was associated with an increased risk of depression. Vitamin D receptors are present in multiple brain regions associated with depressive disorders, including the prefrontal cortex and hippocampus, and cells in many of these regions are capable of metabolizing vitamin D to calcitriol. Animal studies have suggested that vitamin D could increase the synthesis and/or metabolism of neurotransmitters, including dopamine and norepinephrine, though results have been inconsistent [47]. Some cross-sectional and prospective analyses, investigators have observed an inverse relation between blood 25(OH)D levels and prevalent or incident depression. To date, few clinical trials have been conducted to determine the effect of vitamin D supplementation in prevention or treatment of depression and they showed mixed results. A recent large randomised placebo-controlled clinical trial including more than 36,000 postmenopausal women, 2 years of supplementation with 400 IU/day of vitamin D3, combined with 1,000 mg/day of calcium did not influence the risk of depression in postmenopausal women. It was proposed to study the supplementation of higher dose (800 UI) with a lower dose of calcium [47].

In conclusion, beside bone health, vitamin D was shown to play a role in numerous acute and chronic diseases such as:

- **Acute infections (bacterial and viral)**
- **Dermatologic disease (psoriasis)**
- **Autoimmune diseases (Bowel disease, multiple sclerosis, rheumatoid arthritis, ...)**
- **Cardiovascular diseases (including myocardial infarction, ...)**
- **Diabetes**
- **Psychiatric diseases (Dementia, Alzheimer, depression, ..)**

CHAPTER 4

VITAMIN D AND RETINA

Like other tissues, there is evidence of vitamin D target cells in the eye [48] which may influence various ocular pathologies (Table 6). Vitamin D receptors are expressed extensively in the retina, including retinal and choroidal vascular endothelial cells [24]. The vitamin D-dependent calcium binding protein is also expressed by the human retina [49]. Therefore, it was suggested that vitamin D might prevent the development and progression of retinopathy including diabetic retinopathy (DR) and age-related macular degeneration (AMD), as a result of its anti-inflammatory and anti-angiogenic properties [42, 50].

Table 6: Vitamin D and ocular diseases

Vitamin D status	Low serum 25(OH)D levels associated with disease risk; or high 25(OH)D levels associated with decreased disease prevalence	Myopia Age-related macular degeneration Diabetic retinopathy Dry eye syndrome
Genetic variation	Gene polymorphisms associated with ocular disease (VDR, CYP24A1, CYP27B1, DHCR7*)	Myopia Age-related macular degeneration Diabetic retinopathy Uveitis
Treatment/ supplementation	Improvement of disease or pathology with either systemic or local vitamin D treatment	Retinoblastoma (mouse) Choroidal melanoma (mouse) Retinal aging (mouse) Ischemic retinopathy (mouse) Type 2 diabetic retinopathy (rat) Experimental autoimmune uveitis (mouse) Corneal injury (mouse) Corneal transplantation (rat) Corneal neuralgia (human case report) Intraocular pressure (non-human primate)
***In vitro* cell studies**	Expression of vitamin D pathway components and/or biological effect of vitamin D treatment	Corneal epithelial cells Lens epithelial cells Corneal endothelial cells Scleral fibroblasts Non pigmented ciliary body epithelial cells Adult retinal pigment epithelial cells Ganglion cell layer Retinal photoreceptors Retinoblastoma cells (Y79, Weri-RB1)

*DHCR7: 7-dehydrocholesterol reductase; Adapted from Reins & McDermott, 2015 [48]

4.1 VITAMIN D AND DIABETIC RETINOPATHY

Diabetic retinopathy (DR), which is among the most common diabetes complications, is a leading cause of blindness among working-aged adults worldwide [51, 52]. Major risk factors for DR include a longer diabetes duration, poor glycemic control, and hypertension, which have been strongly and consistently associated with diabetic retinopathy across populations [53].

VITAMIN D STATUS IN DIABETIC RETINOPATHY

Several recent cross-sectional studies showed an inverse relationship between 25(OH)D levels in type 1 and type 2 diabetic patients and DR using study designs tempting to attenuate potential confounding factors such as seasons, physical activity, drugs affecting vitamin D or calcium metabolism, or metabolic diseases. These studies showed an inverse graded relationship between serum 25(OH)D levels and the severity of diabetic retinopathy (Table 7).

Table 7: Vitamin D and diabetic retinopathy in observational studies

Ref	Study design	Main results
[43]	Case-control study in diabetic patients Mean age 60 years (65% of men) 1) 144 patients with DR 2) 139 patients without DR	Lower 25(OH)D blood levels in patients with DR (19.2±10.1 *versus* 20.5±8.1 ng/mL, p=0.05) 25(OH)D < 20 ng/mL in 50.7% of patients without DR, 55.6% of patients with DR grade 1, and 67.1% in patients with DR grade 2-4 (p=0.03) Higher risk of DR (x1.43) in patients with severe VDD (<15 ng/mL) (p=0.03)
[54]	Cross-sectional study in 517 adolescents with type-1 diabetes. Mean age 15 years	DR present in 18% of patients with 25(OH)D ≤ 20 ng/mL *versus* 9.0% in patients with 25(OH)D > 20 ng/mL (p<0.02) Higher risk of DR (x2.1) in patients with 25(OH)D ≤ 20 ng/mL (20ng/mL): OR=2.12 (95%CI: 1.03-4.33, p=0.04)
[53]	A population-based cross-sectional study using a nation-wide (Korea), systemically stratified, multistage, clustered sampling method 2,113 diabetic patients aged ≥ 40 years (1,063 men and 1,050 women)	63% lower risk of DR in men with high blood 25(OH)D levels: OR=0.37 (95%CI: 0.18-0.76) 85% lower risk of proliferative DR in men with high blood 25(OH)D levels: OR=0.15 (95%CI: 0.03-0.83) Similar associations were not observed in women

Ref	Study design	Main results
[55]	715 patients with type 2 diabetes: Mean age 68±12 years (61% of women) • 490 patients without DR • 168 patients with non proliferative DR • 57 patients with proliferative DR.	25(OH)D <30 ng/mL in 75.4% of patients (= Hypovitaminosis D) 25(OH)D <20 ng/mL in 36.6% of patients (= Deficiency in vitamin D) Inverse graded relationship between serum 25(OH)D and severity of DR (p=0.003)
[56]	Case-control study in diabetic and non diabetic patients: - 66 diabetic patients - 20 non diabetic healthy subjects	Lower serum concentrations of 25(OH)D2 in diabetic patients (57.3±21.4 pmol/L) compared to non-diabetic patients (89.4±18.0 pmol/L), p<0.001 Lower serum concentrations of 25(OH)D2 in diabetic patients with proliferative DR (43.1±19.5 pmol/L), with pre-proliferative DR (47.7±13.3 pmol/l), compared to background DR (63.4±17.3 pmol/L)
[57]	5 groups of subjects or patients with type 2 diabetes matched for age, sex and race: • 47 subjects without diabetes and eye disease; • 51 patients without diabetes, but with eye disease (uveitis, AMD,...) • 41 patients with diabetes, but no evidence of DR. • 40 patients with non-proliferative DR. • 42 patients with evidence of proliferative DR.	Lower 25(OH)D levels in diabetic patients (22.9 ng/mL) compared to non diabetic subjects (30.3 ng/mL, p<0.001) Lower levels of 25(OH)D levels in patients with proliferative DR (21.1±10.5 ng/mL) compared to patients with non proliferative DR (23.6±10.3%), diabetic patients without DR (24.3±10.3), non diabetic patients with ocular diseases (28.8±14.3), and subjects without diabetes or ocular disease (31.9±12.9 ng/mL)

OR: odds ratio, VDD: vitamin D deficiency

Patients with diabetic retinopathy have lower levels of circulating vitamin D.

Zoppini et al. examined whether there was a significant relationship between serum 25(OH)D levels and the prevalence of diabetic retinopathy in a large cohort (N=715 outpatients) of well-characterised patients with type 2 diabetes [55]. They found that 34% of patients with 25(OH)D levels < 30 ng/mL had retinopathy (any degree) (*versus* 24% in patients with 25(OH)D ≥ 30 ng/mL, p=0.018).

Patients with advanced retinopathy are more frequently vitamin D deficient compared to diabetic patients without DR.

Similarly in Spain, Alcubierre et al. [43] showed that the levels of 25(OH)D was significantly lower in patients with diabetic retinopathy than in patients without retinopathy (p=0.05). The rate of patients with vitamin D deficiency (i.e. 25(OH)D < 20 ng/mL) was 61.9% *versus* 50.7%, respectively. The relative risk to have a diabetic retinopathy was 1.43 (p=0.03) in patients with a 25(OH)D < 15 ng/mL. In multivariate analyses, a serum 25(OH)D level < 15 ng/mL was significantly associated with the presence of

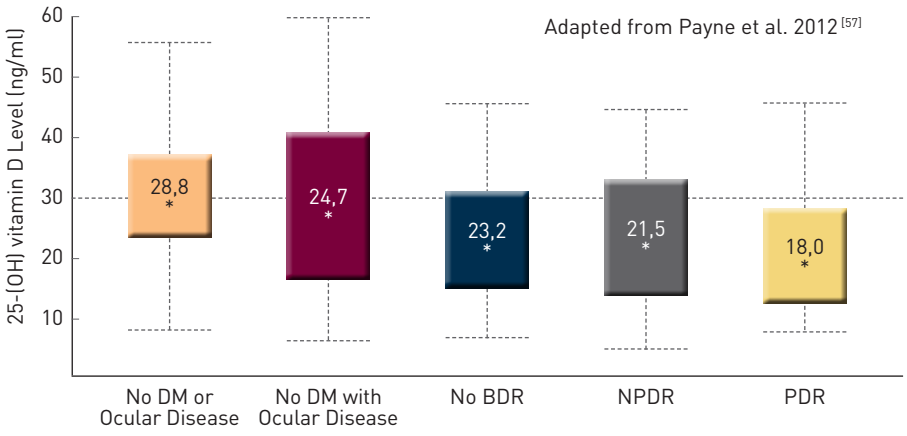
retinopathy (p=0.009) independently of potential predictors including age, sex, race, hemoglobin A1c, creatinine, disease duration, body mass index (BMI), hypolipidaemic treatment, and physical activity. They found that patients with advanced retinopathy (grades 2-4, N=76 patients) were more frequently vitamin D deficient compared with type 2 diabetic patients without retinopathy (p=0.03).

The inverse relationship between vitamin D circulating levels and DR was confirmed more recently in an epidemiologic study performed in Korea [53]. After adjusting for potential confounders, including age, diabetes duration, HBA1c levels, and hypertension, men with high blood 25(OH)D levels had 63% (OR=0.37; 95% CI, 0.18-0.76) lower risks of any DR and 85% (OR= 0.15; 95% CI, 0.03-0.83) lower risk of proliferative DR. This study provides the first epidemiologic findings of the inverse relationships of blood 25(OH)D levels with any diabetic retinopathy and proliferative diabetic retinopathy in men. However, such association was not found in women for reasons which remain to be determined.

Kaur et al. were the first to demonstrate an association between vitamin D deficiency and diabetic retinopathy, independently of diabetes duration and HbA1c in young adolescents with type-1 diabetes [54]. Patients with vitamin D deficiency presented more frequently with diabetic retinopathy (18% *versus* 9%, p=0.02) and had an increased risk of 2.12 (95%CI: 1.03, 4.33).

Diabetic patients with vitamin D deficiency (< 20 ng/mL) have increased risk of proliferative diabetic retinopathy.

Payne et al. [57] performed a study in 221 patients divided into five subgroups based on their diabetes status and retinopathy findings: patients without diabetes and ocular disease (Group 1); patients without diabetes, but some form of ocular disease, such as uveitis or macular degeneration (Group 2); subjects with type 2 diabetes, but no evidence of diabetic retinopathy (Group 3); patients with nonproliferative diabetic retinopathy (Group 4); and patients with evidence of retinal neovascularization on the optic disc, retina, or iris with or without vitreous hemorrhage or prior panretinal photocoagulation (Group 5). In multivariate analysis, there was a statistically significant difference ($p < 0.001$) between groups in 25(OH)D levels with lower levels in patients with proliferative diabetic retinopathy (21.1±10.5 ng/mL) compared to the other groups (Figure 7).



DM: diabetes mellitus; BDR: background retinopathy; PDR: proliferative retinopathy; NPDR: non-proliferative retinopathy.

Figure 7: Inverse graded relationship between serum 25(OH)D level and the severity of diabetic retinopathy

The upper limit of each box represents the 75th percentile while the lower limit represents the 25th percentile. The dotted lines represent the 95th and 5th percentile, and the numerical value is the median for each group.

Low circulating levels of calcitriol (the active metabolite of Vitamin D) was also shown to be directly associated with diabetic retinopathy. In a small study comparing diabetic and non diabetic subjects, Aksoy et al. [56] showed an inverse relationship between the severity of the retinopathy, i.e., neovascularization, and serum $1,25(OH)_2D$ concentrations. The mean calcitriol concentrations fell with increasing severity of the diabetic retinopathy (63.4±17.3 pmol/L for background DR, 47.7±13.3 pmol/L for preproliferative DR, and 43.1±19.5 pmol/L for proliferative DR). Serum 25(OH)D concentrations were also lower ($p < 0.001$) in diabetic patients compared with the control group.

VITAMIN D AND DIABETIC RETINOPATHY PATHOGENESIS

The inverse relationship between low vitamin D status and diabetic retinopathy is consistent with a role of vitamin D in the protection of retinopathy. However, the cross-sectional design of the studies described above limits the ability to assess causality. Thus, it is not possible to determine, from these studies, if the vitamin D insufficiency leads to diabetic retinopathy or if diabetic retinopathy leads to vitamin D insufficiency (e.g. by less outdoors activity, sun exposure time, and thus less skin 25(OH)D production in these patients) [57].

Biological models support a causal role for vitamin D deficiency in proliferative retinopathy [54].

However, there is consistent data from experimental studies indicating that vitamin D may protect against diabetic retinopathy through its anti-inflammatory and anti-angiogenetic properties.

Vitamin D may prevent diabetic retinopathy development and progression by its anti-inflammatory and anti-angiogenetic properties.

Indeed, there is evidence to suggest that vitamin D plays a role in the pathogenesis of diabetic retinopathy through its effects on the immune system. Inflammatory cytokines, such as TNFα, TNFβ, IL-6, and plasminogen activator inhibitor-1 are upregulated in patients with type 2 diabetes, and it has been shown that vitamin D decreases the production of several pro-inflammatory cytokines [30]. Vitamin D also exerts an anti-inflammatory effect by decreasing the proliferation of T-helper cells, T-cytotoxic cells and the generation and activity of natural killer cells [30].

Vitamin D may also contribute to diabetic retinopathy via inhibition of angiogenesis. A recent study found that vitamin D deficiency was associated with vascular endothelial dysfunction in middle aged and elderly adults [58]. Vitamin D was shown to reduce vascular endothelial growth factor (VEGF) expression, endothelial cell proliferation, and platelet-derived growth factor (PDGF) expression [53, 59]. Albert and colleagues showed that the active metabolite of vitamin D, calcitriol, was a potent inhibitor of retinal neovascularization *in vivo* [59]. They also found that calcitriol inhibits retinal endothelial cell capillary morphogenesis *in vitro* [59]. Furthermore, calcitriol downregulates hypoxia-inducible factor-1 (HIF-1) transcriptional activity, as well as HIF-1 target genes, such as VEGF [60]. As several of the complications in diabetic retinopathy, such as macular oedema and neovascularization, are driven by VEGF production [57, 61], vitamin D could exert its positive effect via calcitriol mediated VEGF reduction. In addition, vitamin D also inhibits gene expression of matrix-metalloproteinases (MMPs) which are known to play a role in diabetic retinopathy development [53, 62].

Genetic variations in VDR have also been associated with diabetic retinopathy. In a cohort of Caucasian patients with type 1 diabetes, patients with the FokI VDR polymorphism (FF genotype) had a lower incidence of advanced diabetic retinopathy [50]. The FokI substitution is a functional polymorphism which has been reported to increase immune cell activity and therefore could have a protective effect on DR development. In other studies, the VDR BsmI gene polymorphism was also associated with risk of DR and the Taq I polymorphism with severe DR [48].

A consequence of low circulating vitamin D in diabetic patients may be the development of optic neuropathy through a decrease neuroprotective effect. Recently, in a study comparing patients with early diabetic retinopathy with or without vitamin D deficiency (25(OH)D < 20 ng/mL), it was shown that the mean RNFL thickness was significantly reduced in vitamin D deficient patients compared to non deficient patients (94.6±9.0 µm *versus* 112.7±11.9 µm, $p < 0.001$) [63]. A significant correlation between the mean RNFL thickness and serum 25(OH)D concentrations was observed in vitamin D deficient patients for the right eye ($R=0.74$, $p < 0.001$) and the left eye ($R=0.88$, $p < 0.001$). This study suggests that vitamin D functions as a neuroprotective component for optic nerves in patients with early-stage diabetic retinopathy and that low serum 25(OH)D concentrations contribute to retinal nerve fiber layer (RNFL) thinning in early stage DR patients with Vitamin D deficiency.

Obviously, vitamin D may also play a protective role through its effects on glycemic control and hypertension, both significant risk factors for the development and progression of diabetic retinopathy [30, 64].

In conclusion, observational studies indicate that the risk of diabetic retinopathy is associated with low levels of circulating 25(OH)D and advanced DR is increased by vitamin D deficiency. Although causality of vitamin D cannot be implied from these cross-sectional studies, biological effects of vitamin D (including anti-inflammatory effects, inhibition of angiogenesis and apoptosis process) are consistent with a role of vitamin D in the development of diabetic retinopathy. Well-designed prospective observational studies are needed to confirm the role of vitamin D status in the development of diabetic retinopathy [43].

4.2 VITAMIN D AND AGE-RELATED MACULAR DEGENERATION

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness in elderly population. AMD is a degenerative retinal disease that affects the photoreceptors, the retinal pigmentary epithelium (RPE), the Bruch's membrane, and the choroid [64b]. Early AMD is characterised by subretinal deposits, known as drusen, that measure greater than 63 μm and hyper- or hypopigmentation of the RPE [65]. Intermediate AMD is characterised by the accumulation of focal or diffuse drusen measuring greater than 125 μm and hyper- or hypopigmentation of the RPE. Advanced AMD can be classified into either of two categories. The first of these comprises geographic atrophy (i.e., dry, or non-exudative, AMD), which is characterised by a sharply delineated area of RPE atrophy measuring at least 175 μm along one dimension and including visible choroidal vessels. The alternative form of the advanced disease is choroidal neovascularization (i.e., wet or exudative AMD), which may involve some or all of the following: subretinal neovascular membranes; subretinal fluid, exudates, and haemorrhages; pigment epithelial detachment; and subretinal/intraretinal scarring. Advanced AMD can result in loss of central visual acuity and lead to severe and permanent visual impairment and blindness. Although neovascular age-related macular degeneration represents only 10 to 15% of the overall prevalence of age-related macular degeneration, it is responsible for more than 80% of cases of severe visual loss or legal blindness (i.e., visual acuity of 20/200 or worse) resulting from age-related macular degeneration [67].

The pathogenesis of AMD is not well known but is likely to involve a complex interaction of multiple factors, including light damage, oxidative stress, inflammation, possible disturbance in the choroidal blood vessels, and genetic predisposition [68]. AMD can be caused by interaction of various environmental and genetic factors [70, 71]. This includes advanced age, sex (higher in women), race (higher in Caucasian, and lower in black), tobacco use, genetic factors, and an antioxidant-deficient diet. Other factors that have been reported to influence risk for AMD include sunlight exposure, alcohol consumption, increased plasma fibrinogen levels, diet, hypertension, BMI, and iris colour [71]. Regarding diet, antioxidants such as carotenoids, zinc, and vitamins A and E may provide a protective benefit against AMD [71, 72]. Some study showed a significant association ($p < 0.001$) between dietary intake of carotenoid (lutein/zeaxanthin) and β-carotene with the reduction in risk for AMD [66, 73]. Recently, much progress has been made in gene discovery and mechanistic studies, which clearly indicate that AMD involves the interaction of multiple genetic and environmental factors [71].

Progress made in gene discovery and mechanistic studies indicate that AMD involves the interaction of multiple genetic and environmental factors.

VITAMIN D STATUS IN AMD PATIENTS

Association between vitamin D status (as assessed by the serum 25(OH)D) and the risk of early or late AMD have been reported in several cross sectional studies performed since a decade. High levels of 25(OH)D (generally above 30 ng/mL, i.e. 75 nmol/L) have been associated with decreased odds of early or advanced AMD in various different populations (Table 8) [68, 74-77]. By contrast Cougnard et al. did not show statistically significant relationships between vitamin D status and early or late AMD in a cohort of

963 community-dwelling elderly in France (The ALIENOR study) [78]. Golan et al. did not find significant association between vitamin D and the prevalence of AMD in another cross-sectional study including a large, representative sample (N=9,167) of the Israeli population aged 60 years or older [79]. Beside the retrospective and cross-sectional design, all these studies have their own limitations which may explain such inconsistency across studies including lack of statistical power, AMD staging misclassification, confounding factors including sunlight exposure, country, region, nutritional factors, and other environmental factors. Two recent meta-analysis were performed recently [79b, 79c]. For Annweiler et al. [79a], there is evidence that high blood 25(OH)D may be protective against AMD, especially late AMD. On the opposite, Wu et al. concluded that there is no evidence to indicate an inverse association between serum vitamin D levels and any stages and subtype of AMD risk [79c]. Thus, the association between vitamin D status and AMD is inconsistent and needs additional assessments [80].

Table 8: Association of vitamin D and AMD in observational studies

Ref	Study design	Main results
[74]	Cross-sectional study in noninstitutionalised population in the USA 7,752 individuals (age > 40 years)	Compared to patients with serum 25(OH)D < 17 ng/mL, patients with 25(OH)D > 34 ng/mL had: • A 36% lower risk of early AMD (OR=0.64, 95%CI: 0.5-0.8, p<0.001) • A 24% lower risk of soft drusen (OR=0.76, 95%CI: 0.60-0.96, p=0.006)
[68]	Subpopulation of the WHIOS study 1,313 female subjects	48% lower risk of early AMD in women aged <75 years with serum 25(OH)D > 30 ng/mL (OR=0.52, 95%CI: 0.29-0.91, p=0.02)
[75]	National cross-sectional study 17,045 male or female individuals (age ≥ 40 years)	In men, 68% lower risk of late AMD in subjects with serum 25(OH)D > 24 ng/mL) (OR=0.32, 95%CI: 0.12- 0.81, p=0.018)
[76]	Retrospective case-control study • 216 patients with NNV AMD • 146 patients with NV AMD • 100 patients without AMD	65% lower risk of NV AMD in patients with serum 25(OH)D > 40 ng/mL (OR=0.35, 95%CI: 0.18-0.68, p=0.0073)
[77]	Study population derived from the NAS-NRC World War II Veterans Twin Registry Caucasian male monozygotic twin pairs with discordant phenotype for: • stage of AMD (28 pairs) • drusen area (60 pairs) • druzen size (40 pairs) • increased pigment area (56 pairs)	Higher dietary intake of vitamin D in twins with less severe AMD (p=0.01) and small druzen (p=0.05)

OR: adjusted odds ratio; NAS-NRC: National Academy of Sciences-National Research Council; WHIOS: Women Health Initiative Observational Study

Vitamin D deficiency is associated with higher risk of early AMD and soft drusen.

In 2007, Parekh et al. were first to suggest a role of vitamin D in AMD. They showed a significant correlation between reduced serum vitamin D levels and risk of early AMD in a cross-sectional study involving subjects of the third National Health and Nutrition Examination Survey (NHANES III) [74]. As a reminder, the NHANES III is a national survey, in a representative stratified probability sample of the noninstitutionalised civilian population in the United States. The survey was conducted from 1988 through 1994 and includes a sample of approximately 40,000 persons (from 2 months of age). Data were collected in 2 phases over a 6-year period between 1988 and 1994. Oversampling of non-Hispanic black individuals, Mexican American individuals, and adults aged 60 years and older was done to allow more accurate estimates for these individual subgroups [81]. Of the targeted 14,464 participants aged 40 years and older who were eligible for the survey, 11,448 persons (79%) were interviewed. The final analysis included 7,752 individuals (53% of targeted sample) and consisted of non-Hispanic white (n=3,889), non-Hispanic black (n=1,820) and Mexican American individuals (n=1,742) and people of other races and ethnicities (n=301). They all had gradable non mydriatic fundus photographs, serum vitamin D data, and serum cotinine levels (a biomarker for smoking status) [74].

The objective was to evaluate the associations between levels of 25(OH)D in serum and prevalent AMD. These associations were adjusted for potential confounder including age, BMI, personal history of cardiovascular disease (stroke, heart attack, or angina), hypertension, diabetes mellitus, serum cotinine level, alcohol consumption, C-reactive protein level, fibrinogen level, and levels of dietary lutein and zeaxanthin, zinc, and vitamin E. In this study, the odds ratio (OR) and 95% CI for early AMD among participants in the highest (5th quintile of 34 ng/mL) *versus* the lowest (1st quintile of 17 ng/mL) serum 25(OH)D was 0.64 (95% CI, 0.5-0.8; $p < 0.001$). The association with soft drusen was also statistically significant (adjusted OR: 0.76; 95%CI, 0.60-0.96; p=0.006). However, there were no associations between vitamin D serum levels and pigmentary abnormalities or advanced AMD, likely due to the small sample population (185 and 54 patients respectively). Milk intake (fortified in vitamin D in the USA) was inversely associated with early AMD (OR: 0.75; 95% CI, 0.6-0.9) and fish intake inversely associated with advanced AMD (OR: 0.41; 95% CI, 0.2-0.9). The association observed between low vitamin D status and risk of early AMD were confirmed in patients not taking milk or fish, as well as in patients not taking multivitamin supplements [74].

THE CARED STUDY

Vitamin D may have a protective effect in early AMD in patients aged < 75 years.

The relationship between serum 25(OH)D concentrations and the prevalence of early AMD was investigated among participants of the Carotenoids in Age-Related Eye Disease Study (CAREDS) [68]. The CAREDS population consists of women (50-79 years) who were enrolled in 3 investigating sites of the Women Health Initiative Observational Study (WHIOS). Participants with baseline WHIOS intakes of lutein plus zeaxanthin above the 78th and below the 28th percentiles (assessed in 1993-1998) were recruited. Of the 3,143 women who fulfilled these criteria, 1,313 (mean age 69±0.4 years) accepted to participate, had gradable fundus photographs (taken from 2001-2004), and had serum vitamin D (assessed in 1993-1998). The associations were estimated by quintile of the 25(OH)D distribution.

The results showed that participants with high (> 34 ng/mL, quintile 5) compared to low vitamin D (< 12 ng/mL, quintile 1) status were more likely to be Non-Hispanic White, had a higher income, consumed more alcohol, were engaged in a higher level of recreational physical activity, reported greater ocular visible sun exposure, had a family history of AMD, had a lower BMI, were less hypertensive, and had lower levels of C-reactive protein. Participants with high vitamin D status were also more likely to have higher calorie consumption, lower intake of fat, greater fiber intake, and greater intake of antioxidant nutrients. They consumed a greater number of fruit, milk, and fortified cereal servings, had higher scores on the Healthy Eating Index (HEI) 2005, and were more likely to use supplements compared to individuals with low vitamin D status. There were 241 cases of early AMD, and only 26 cases of advanced AMD (1046 women had no evidence of AMD). The analyses showed that, overall, there was no significant relationship between vitamin D status and early or advanced AMD. However, the association was shown to be modified by age with a strong interaction (p=0.0025). In the multivariate models adjusting for AMD risk factors, women < 75 years (N=968) with the highest 25(OH)D concentrations (above 75 nmol/L i.e. 30 ng/mL) had 48% decreased odds of early AMD (Table 9). Similarly, the odds of pigmentary abnormalities was 57% lower (OR 0.43, 0.18–0.96; p=0.02; for quintile 5 vs. 1). These associations in women < 75 years were attenuated after adjustment for BMI and physical activity in the multivariate model, which may be explained by the strong association between 25(OH) levels with these variables. The results of this study are in line with the previous study suggesting a protective effect of vitamin D in early AMD [74]. However, this needs to be confirmed in further prospective studies.

Table 9: Association between vitamin D and early AMD according to age

	QUINTILE OF SERUM 25(OH)D DISTRIBUTION (NG/ML)					
	Quintile 1	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	
Median	**12.2**	**17.6**	**22.4**	**26.8**	**34**	**P for trend***
Range	**(2.8-15.2)**	**(15.3-20)**	**(20.4-24.4)**	**(24.8-30)**	**(30-66)**	
Women ≥ 75 years (N=319)						
OR	1	1.10	1.52	1.55	1.76	0.05
[95% CI]	-	[0.48; 2.57]	[0.66; 3.60]	[0.69; 3.58]	[0.77; 4.13]	
Women < 75 years (N=968)						
OR	1	0.50	0.66	0.58	**0.52**	0.02
[95% CI]	-	[0.28; 0.87]	[0.38; 1.12]	[0.33; 1.01]	**[0.29; 0.91]**	

Multivariate model adjusting for smoking pack years, iris pigmentation, family history, cardiovascular disease, diabetes and hormone use status. Adapted from Millen et al. 2011 [68]

*A*P*-value was obtained for the linear trend by replacing the categorical predictor with the continuous variable (serum vitamin D)

Vitamin D may play a key role in the inhibition of late AMD development.

In a Korean study (KNHANES), Kim et al. showed that high level of blood 25(OH)D was inversely associated with late AMD in men [75]. This was a national cross-sectional study conducted in 17,045 participants aged 40 years and older (mean age 47.9 years; 62% of participants were males). Retinal examination was done under physiological mydriasis and AMD graded according to the international classification of grading system. Overall, 1,163 participants showed early AMD and 115 participants had late AMD (defined as the presence of wet or dry AMD). Subjects with AMD were more likely to be older or smokers, to have hypertension, and longer sun exposure than those without AMD. As blood 25(OH)D increased, participants were more likely to be male and to have longer sun exposure. After adjusting for potential confounders including age, sex, smoking status, hypertension, heart problems, stroke, and sunlight-exposure time, the odds ratio for late AMD significantly decreased in the highest blood vitamin D status quintile (25(OH) D > 24.3 ng/mL) with an OR of 0.32 (95% CI: 0.12-0.81; P for trend = 0.018) compared with the lowest quintile (< 13.1 ng/mL) in men, but not in women (Table 10). In this study, early AMD was not associated with blood 25(OH)D levels. These findings suggest that vitamin D may play a key role in the inhibition of late AMD development. However, it is unclear why the vitamin D status is associated with late AMD in men but not in women. Thus, further studies are necessary to elucidate sex-specific mechanisms.

Table 10: Association between vitamin D status and late AMD according to gender

	QUINTILE OF SERUM 25(OH)D DISTRIBUTION (NG/ML)					P for trend*
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
Range	**< 13.1**	**13.1 – 16.4**	**16.4 – 19.7**	**19.7 – 24.3**	**> 24.3**	
Males (N=7367)						
OR	1	0.46	0.44	0.40	**0.32**	0.018
[95% CI]	-	[0.16; 1.28]	[0.18; 1.06]	[0.16; 1.01]	**[0.12; 0.81]**	
Females (N=9678)						
OR	1	0.91	0.72	2.60	1.90	0.059
[95% CI]	-	[0.29; 2.87]	[0.23; 2.23]	[0.86; 7.85]	[0.66; 5.44]	

*Multivariate model adjusting for age, smoking, hypertension, heart problem, stroke, and sun exposure. Adapted from Kim et al. 2014 [75]

Vitamin D deficiency has been associated with the development of neovascular AMD.

A recent retrospective study compared the vitamin D status among patients with neovascular AMD (NV AMD), non neovascular AMD (NNV AMD), and control patients [76]. Overall, 216 patients with NNV AMD, 146 patients with NV AMD, and 100 control patients without AMD were included. The levels of 25(OH)D (mean ± SD) were significantly lower in NV AMD patients (26.1 ± 14.4 ng/mL) than in NNV AMD patients (31.5 ± 18.2 ng/mL, p=0.003) and control patients (29.4 ± 10.1 ng/mL, p=0.049). The prevalence of vitamin D insufficiency (25(OH)D < 30 ng/mL), deficiency (< 20 ng/mL), and severe deficiency (< 10 ng/mL) were highest in the NV AMD group (Figure 8). The highest quintile of 25(OH)D was associated with a 65% lower odds (OR=0.35, 95%CI: 0.18-0.68) for NV AMD. The authors conclude that it is biologically plausible that active vitamin D may affect the development of NVAMD more than that of NNVAMD due to its antineovascular properties.

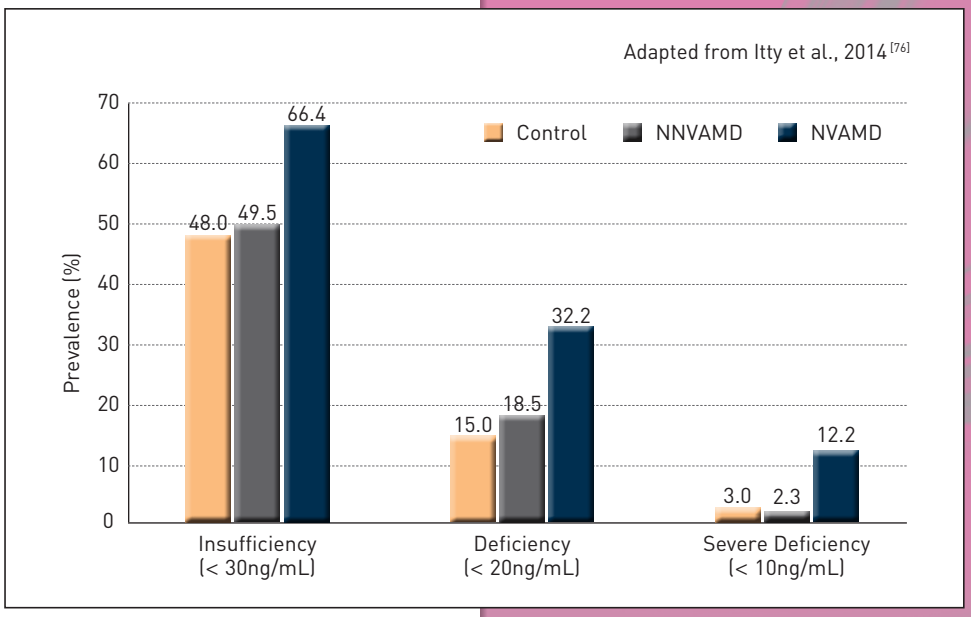


Figure 8: Vitamin D status in patients with neovascular and non neovascular AMD

The prevalence of vitamin D insufficiency, deficiency, and severe deficiency were all highest in the neovascular AMD (NVAMD) *versus* non neovascular AMD (NVVAMD) and control patients.

High dietary intake of vitamin D has been associated with less severe AMD and smaller drusen size in homozygotic twins.

Because monozygotic twins are genetically identical, they are considered ideal experimental models for studying the role of environmental factors as determinants of complex diseases and phenotypes. To assess the association between vitamin D intake and AMD, Seddon et al. carried out a study in Caucasian male monozygotic twin pairs with discordant AMD phenotype [77]. Twin pairs discordant were selected in each of the following phenotypic categories: Stage of AMD (n=28), drusen area (n=60), drusen size (n=40), and increased pigment area (n=56). Dietary intake of vitamin D was assessed using a food frequency questionnaire and ocular characteristics based on fundus photographs using the Wisconsin Grading System and the 5-grade CARMS. The study showed that higher dietary intake of vitamin D was present in the twins with less severe AMD (p=0.01) and smaller drusen size (p=0.05) compared with co-twins, adjusted for smoking and age. Their findings suggest that some nutritional factors (including vitamin D) could be involved in the aetiology of AMD, in addition to genetic susceptibility.

Higher dietary intake of vitamin D could contribute to a reduced risk of neovascular AMD in elderly patients.

Consistently, in a recent Japanese case-control study, patients aged 50 years or older with neovascular AMD (N=161) and control subjects (N=369) randomly selected from the population aged ≥ 65 years were assessed using a brief-type self-administered questionnaire (BDHQ) on diet history including 58 food and beverage items. Logistic regression analysis adjusted for smoking, age, sex, chronic disease, supplement use, and alcohol consumption demonstrated that low intakes of vitamin D were significantly (p=0.002) associated with neovascular AMD (Table 11), together with other nutrients including n-3 fatty acid, alpha-tocopherol, zinc, vitamin C, and beta-carotene [82].

Table 11: Association between vitamin D intake and neovascular AMD

	QUINTILE OF SERUM 25(OH)D DISTRIBUTION (NG/ML)					
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P for trend*
Range	≤ 12.2	12.3 – 15.9	15.9 – 20.4	20.4 - 27.3	> 27.3	
N of cases/ controls	46/59	33/72	29/77	24/81	25/80	0.002
OR	1	0.6	0.5	0.40	0.4	
[95% CI]	-	[0.3; 1.1]	[0.3; 0.9]	[0.2; 0.7]	[0.2; 0.8]	

*Multivariate model adjusting for smoking history, age, sex, chronic disease history, supplement use, and alcohol consumption. Adapted from Aoki et al. 2016 [82]

There is an inverse relationship between vitamin D insufficiency and reduced macular thickness.

Whether vitamin D is causal or consecutive to the development of AMD is still a matter of intense research and debate. However a recent study performed by Graffe et al. highlighted an association between vitamin D insufficiency (25(OH)D < 50 nmol/L) and subclinical macular changes, thus reinforcing the hypothesis of an inverse impact of vitamin D insufficiency on the retina [83]. In this study, 62 French relatively healthy older community-dwellers with no patent macular dysfunction (mean age ± SD: 71.2±5.0 years; 45.2% of women) included in the Gait and Alzheimer Interaction Tracking (GAIT) study were separated into two groups according to their serum 25(OH)D level (i.e., insufficient < 50 nmol/L or sufficient ≥ 50 nmol/L). The macular thickness was measured on 1,000 µm central macula with optical coherence tomography (OCT), and further binarised according to normal values of macular thickness (i.e., 267.74 µm for males, and 255.60 µm for females). Age, sex, number of comorbidities, cognitive disorders, BMI, mean arterial pressure, visual acuity, intraocular pressure, serum calcium concentration and season of testing were considered as potential confounders. Results showed a mean serum 25(OH)D concentration of 61.2±26.3 nmol/L (24.5±10.5 ng/mL). Patients with vitamin D insufficiency had a reduced macular thickness compared to those without

(232.9±40.4 μm vs. 253.3±32.1 μm, p=0.042) (Figure 9). After adjustment for potential confounders, vitamin D insufficiency was associated with a decreased macular thickness (coefficient of regression β=-59.4 μm, p=0.001). Consistently, the participants with vitamin D insufficiency had a 3.7-fold higher risk of having abnormally low macular thickness compared with those with sufficient 25(OH)D level (p=0.042). The inverse relationship between vitamin D insufficiency and reduced macular thickness among older patients with no patent macular dysfunction implies that vitamin D insufficiency may be involved in macular thinning.

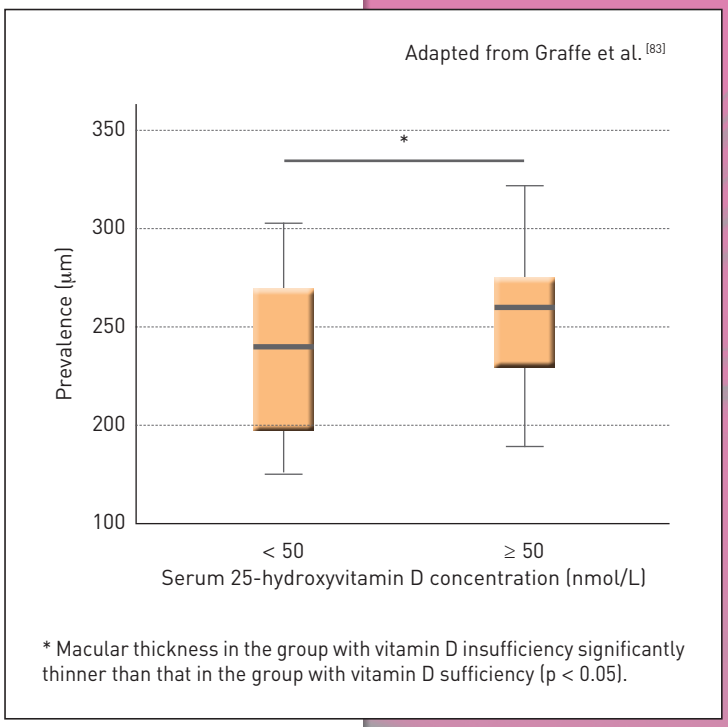


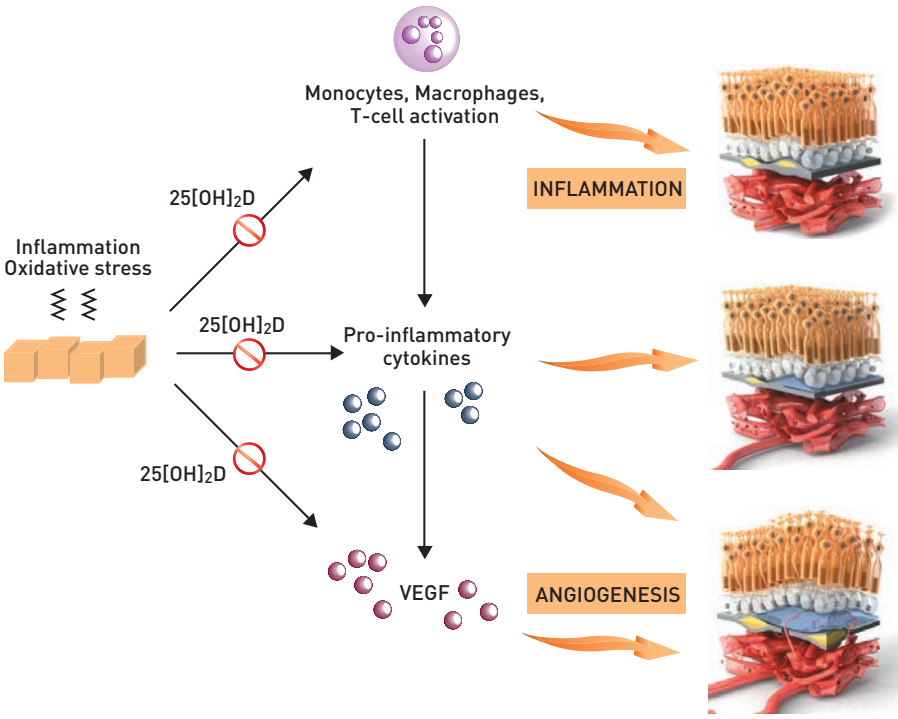
Figure 9: Macular thickness according to serum 25(OH)D concentration in healthy elderly

VITAMIN D AND AMD PATHOGENESIS

Accumulating data from experimental studies suggest that vitamin D may prevent the development of the AMD, by inhibiting two pathogenic pathways: inflammation and angiogenesis as illustrated in Figure 10.

Figure 10: Hypothetical mechanism of vitamin D in AMD pathogenesis

Oxidative stress, produced by aging and other AMD risk factors including smoking, augmented BMI, phototoxicity induce inflammatory reaction, consisting in the production of inflammatory related molecules, recruitment of macrophages, complement activation, microglial activation. Radical oxygen species augmentation can also trigger angiogenic signalling that has a crucial role in the occurrence of the more severe complication of AMD, i.e. choroidal neovascularization [84]. Vitamin D ($25(OH)_2D$) may prevent the development of AMD by inhibiting the progression of inflammation and angiogenesis.



from http://www.scienceofamd.org

Enzymes responsible in vitamin D metabolism and the vitamin D receptor are expressed in human retina and choroid.

A possible role of vitamin D in ocular functioning is supported by evidence that the vitamin D receptor (VDR) and the enzymes involved in the metabolism of vitamin D (CYP27B1, CYP27A1, and CYP24A1) are expressed in the retina and choroid (Table 12). The presence of VDR was first demonstrated by immunohistochemical staining in the inner and outer segment of retinal photoreceptors, and in the ganglion cell layer, the inner nuclear layer, and the retinal pigment epithelium [85]. The presence of the VDR gene in resting retinal and choroidal endothelial cells of human cadaver donors was then confirmed by molecular biology [24]. Recently, the presence of vitamin D hydroxylases (CYP27B1, CYP27A1, CYP2R1, and CYP24A1) was shown in an adult retinal pigment epithelial cell line (ARPE19), suggesting that these cells have the machinery to convert vitamin D and 25(OH)D and $25(OH)_2D$ [86]. Morrison et al. assessed the vitamin D pathway-associated genes in a whole transcriptome analysis of 126 RPE-choroid and 118 retina punches collected from the macular and extramacular regions of eyes derived from 66 human donors [87]. VDR and CYP27A1 expression was detected in the RPE-choroid and CYP27A1 and CYP27B1 in the retina. CYP27A1 was expressed at low to moderate levels, with significant differences detected between the extramacula and macula regions in both non-AMD and

AMD RPE-choroids. Similar macula:extramacula expression differences were observed for CYP27A1 and CYP27B1 in the retina. This suggests that the vitamin D metabolic gene VDR and CYP27B1 may function differently between the macula and extramacula, whether diseased or not. They reported that the VDR expression in many RPE-choroid tissues was at or below the level of detection, but was observed at significantly higher levels in several donors, concomitant with an increased expression of genes involved in local inflammatory responses. There was a trend of slightly higher VDR expression in the macular region of both normal and diseased RPE-choroids (1.3- and 1.5-fold, respectively) which were statistically significance (p = 0.02) in the diseased RPE-choroids, but not in the retina.

Table 12: Expression of vitamin D associated genes in the choroid and retina

• Expression of the VDR in the retina and choroid demonstrated by immunochemistry [85]
• Expression of VDR gene in resting retinal and choroid endothelial cells confirmed by molecular biology [24]
• Expression of vitamin D hydroxylases mRNA and protein in retinal pigment epithelial cell lines [86]
• Detection of vitamin D associated genes in human RPE-choroid and retina [87]

Vitamin D may supress the cascade of destructive inflammation that occurs at the level of the retinal pigment epithelium-choroid interface in early stages of AMD [68].

Although AMD is generally not considered a classic inflammatory disease, immunocompetent cells, such as macrophages and lymphocytes are present in the chorioretinal tissues affected by AMD [84]. It is now recognised that inflammatory events, such as complement activation, immune cells recruitment, and proinflammatory cytokines release, play a role in the development of AMD [48]. Immune components, including immunoglobulins, complement factors, and fibrinogen, have been observed to be entrapped within drusen [74]. Vitamin D, because of its anti-inflammatory, immune-modulating properties may suppress the cascade of destructive inflammation that occurs at the level of the RPE-choroid interface in early stages of AMD. It is known that the VDR is expressed on cells of the human immune system including monocytes/macrophages, T lymphocytes, and dendritic cells [29] and $1,25(OH)_2D$ was shown to suppress proinflammatory cytokines *in vitro*, in part by altering T-cell function toward a Th2 (anti-inflammatory) rather than a Th1 (proinflammatory) response [25]. Vitamin D exerts an anti-inflammatory effect by decreasing the proliferation of lymphocytes, and the generation and activation of natural killer cells, and several proinflammatory cytokines [74]. Studies have reported that vitamin D decreases proliferation of T helper cells, T cytotoxic cells, and natural killer cells and enhances T suppressor cell activity. Vitamin D also decreases the production of proinflammatory cytokines (IL-2, IL-6, IL-8, IL-12) [74].

In a study using aging mice, Lee et al. demonstrated that subcutaneous treatment with vitamin D significantly reduced signs of retinal inflammation. Treated mice for only 6 weeks had fewer macrophages in the subretinal space, less complement (C3b) deposition on Bruch's membrane, and a reduction in retinal amyloid beta accumulation [88]. Since excess of amyloid beta deposition and inflammation are risk factors leading to AMD, this study suggest that vitamin D may reduce retinal inflammation in AMD pathogenesis.

Table 13: Potential anti-inflammatory effects of vitamin D in the retina

• Inhibition of macrophage activation
• Suppression of T-cell activation
• Suppression of proinflammatory cytokines [57, 74]

Vitamin D has been shown to inhibit angiogenesis and fibrosis which are the most critical characteristics of late AMD [75].

In vitro studies performed by Mantel et al. showed $1,25(OH)_2D$ inhibits specific stages of the angiogenic process in a dose dependent manner [89]. The direct effects of $1,25(OH)_2D$ on endothelial cell proliferation and morphogenesis observed with the use of these *in vitro* model systems provide evidence consistent with the antiangiogenic effects of $1,25(OH)_2D$ shown *in vivo*. Furthermore, the induction of apoptosis specifically within the activated, angiogenic, endothelial cell population suggests that this may be the mechanism behind the observed antiangiogenic effects of $1,25(OH)_2D$ [89].

Vitamin D may also inhibit angiogenesis and thus the progression from early to neovascular AMD by reducing the expression of VEGF and platelet-derived growth factor (PDGF), and reducing endothelial cell proliferation as shown in cultured endothelial cells, and within animal models of retinoblastoma and oxygen-induced ischemic retinopathy [59, 61, 68, 89].

These results suggest that $1,25(OH)_2D$ (or analogues of this hormone) may be used in the prevention of conditions involving pathological angiogenesis and may also be of use in the therapeutic regression of such conditions characterised by aberrant angiogenesis [89].

In addition to its antiangiogenic effects, vitamin D is also a potent inhibitor of fibrosis. As shown in other tissues [90, 92]. Singh et al. showed that the presence of subretinal fibrosis in patients with wet AMD was associated with a statistically significant lower concentration of 25(OH)D, probably because of its capability to inhibit angiogenesis, fibrosis, inflammation, and oxidation [93]. A total of 178 patients with AMD were included in this cross-sectional single-centre study. They were classified according to the Clinical Age-Related Maculopathy Staging (CARMS) and the presence or not of subretinal fibrosis. No statistically significant difference in the serum level of 25(OH)D was shown across CARMS group 1 to 5. However, among patients with CARMS 5, the presence of subretinal fibrosis was associated with significantly lower concentrations of 25(OH)D as compared to the absence of subretinal fibrosis

(47.2 *versus* 75.6 nmol/L, $p < 0.001$). Patients in CARMS 5 (the most advanced stage) with subretinal fibrosis were more likely to have insufficient levels of 25(OH)D compared to patients without subretinal fibrosis (p=0.006). This relationship was maintained after adjusting for confounding factors such as age, smoking, vitamin D supplementation, sex, physical exercise, and four single nucleotide polymorphisms (SNPs) known to influence systemic vitamin D concentrations.

The anti-fibrotic activity of vitamin D may be directly related to the inhibition of TGFβ activity as shown in other fibrotic diseases. TGFβ is a cytokine that is overexpressed in fibrotic tissues. This is a key regulator of cell growth, differentiation, inflammation, apoptosis, and tissue remodelling leading to tissue fibrosis [90-92]. In patients with bone marrow fibrosis, the serum levels of TGFβ were shown to be inversely associated with the vitamin D status in multivariate analysis [91].

Furthermore, vitamin D was also shown to inhibit matrix metalloproteinase-9 (MMP-9). Metalloproteinases are endopeptidases that can degrade all classes of extracellular matrix proteins. They are involved in tissue remodelling [94] and play a role in choroidal neovascularization [95]. In a cross-sectional study in Indian healthy subjects, vitamin D deficiency was associated with abnormal increases in circulating MMP-9 [62]. Using a keratinocyte cell line, calcitriol was shown to down-regulate the production of MMP-9 induced by TNFα [95].

Table 14: Potential mechanisms of anti-angiogenesis effects of vitamin D in the retina

• Vitamin D inhibits angiogenesis *in vitro* and *in vivo* [89]
• Vitamin D reduces the expression of VEGF and PDGF and endothelial cell proliferation [59]
• Vitamin D inhibits MMP-9 which plays a key role in choroidal neovascularisation [75]
• Vitamin D specifically induces apoptosis of endothelial cells *in vitro* [89]
• Vitamin D is a potent inhibitor of TGFβ and fibrosis [91, 93]

VITAMIN D AND GENE POLYMORPHISM IN AMD

A genetic link exists between the risk of AMD and vitamin D metabolism [87].

Genetic factors are strong determinants for the development and progression of AMD [77]. Some gene polymorphisms have been shown to be strongly associated with AMD susceptibility. These include genetic variations at two major loci on chromosome 1 and chromosome 10 and AMD. Polymorphisms in complement factor H (CFH) in the 1q32 region have been associated with increased risk of all forms of AMD, pointing to the complement pathway and mechanisms related to immunity/inflammation as important factors in AMD pathogenesis. Similarly, variation in the HTRA1 and ARMS2 genes on 10q26 has also been associated with increased AMD risk, confirming the role of extracellular matrix regulation and oxidative stress in this disease [70, 71].

Several recent studies confirmed the interrelationship between gene polymorphism and vitamin D metabolism (Table 15).

Table 15: Vitamin D and increased odds of AMD

• Genetic polymorphism in the VDR and CYP24A1 genes are associated with the risk of AMD[87]
• Significant interaction between vitamin D status and the polymorphism Y402H of the CFH gene has been demonstrated [96]
• A HTRA1 promoter gene polymorphism associated with increased risk of AMD was shown to modify the response of vitamin D and thus extracellular matrix formation and angiogenesis [48]

Genetic polymorphisms in vitamin D pathway genes have been associated with an increased risk for neovascular AMD.

A recent study was performed to assess if genetic variation within the vitamin D metabolism genes might be associated with neovascular AMD [87]. Genotyping was performed with tagging SNPs from the vitamin D metabolism genes (CYP27B1, CYP27A1, CYP24A1, and VDR) in order to find significantly associated AMD risk variants and haplotype within a cohort comprising extremely discordant sibling pairs (i.e. where one sibling has the neovascular form of AMD and the other sibling had no signs of AMD and was older than 65 years). The cohort included 135 subjects and initial findings were then validated and replicated in three additional populations. Several SNP variations within the CYP24A1 genes were found to be significantly associated with neovascular AMD. It was found that single point variants in CYP24A1 (the gene encoding the catabolising enzyme of the vitamin D pathway) influence AMD risk after controlling for smoking history, sex and age. This was the first report demonstrating a genetic association between vitamin D metabolism and AMD risk.

Vitamin D attenuates the increased odds of AMD in post-menopausal women with the Y402H polymorphism in both alleles of the CFH gene.

Several studies have confirmed that the risk of developing AMD is associated with an allele of the complement factor H (CFH) (an inhibitor of the complement alternative pathway) in which a histidine residue is encoded in place of a tyrosine residue at amino acid position 402 (Y402H polymorphism). The increased risk ranges between 2- to 4-fold for heterozygote carriers and 3- to 7-fold for homozygotes [71]. Recently, the association between vitamin D status and AMD according to the CFH Y402H polymorphism was investigated in the Carotenoids in Age-Related Eye Disease Study (CAREDS) (an ancillary study of the Women's Health Initiative) [96]. Both women with inadequate or adequate vitamin D status

(25(OH)D < 12 ng/mL and > 30 ng/mL, respectively) and two risk alleles had an increased odds of AMD compared to non carriers with a vitamin D level > 30 ng/mL (Table 16). Thus, the odds of AMD were highest in subjects with deficient vitamin D status and two risk alleles for the CFH, suggesting a synergetic effect between vitamin DE status and the complement cascade protein function.

Table 16: Risk of AMD in patients with Y4022H polymorphism in two alleles of the CFH gene according to vitamin D status

	25(OH)D SERUM LEVEL			
	<12 ng/mL	12 - 20 ng/mL	20 - 30 ng/mL	P for trend
OR [95% CI]	6.7 [1.6; 28.2]	4.4 [1.3; 14.1]	2.1 [0.6; 7.0]	0.02

Odds ratio (OR) were calculated relatively to the prevalence of AMD among noncarriers and a vitamin D level ≥ 30 ng/mL. OR were adjusted for age, smoking, iris pigmentation, cardiovascular diseases, diabetes diseases, and hormone use status. Adapted from Millen et al. 2015 [96].

A promoter gene polymorphism associated with increased risk of neovascular AMD is modulated by vitamin D.

A single nucleotide polymorphism in the promoter of the HTRA1 (High-temperature requirement factor A1) gene has been associated with a population attributable risk of 49.3% and a 10-times greater risk of developing choroid neovascularization [97, 98]. HTRA1 is a serine protease and a key modulator of proteoglycans degradation in the extracellular matrix. HTRA1 proteolytic activity permits other degradative enzymes, such as collagenases and matrix metalloproteinases, to access their respective substrates. Similar to complement factors, HTRA1 is expressed in drusen of AMD patients. Excessive accumulation of HTRA1 in drusen could compromise Bruch's membrane integrity, thereby allowing expansion of choroidal capillaries and resulting in neovascular AMD. HTRA1 is also a potent inhibitor of TGF-β, which is involved in extracellular matrix formation and angiogenesis and thus represents another potential pathway to AMD. Alternatively, HTRA1-mediated destabilization of Bruch's membrane could contribute to RPE atrophy and geographic atrophy [71]. In rhesus monkey, the HTRA1 promoter region was shown to contain nine VDR binding sites [48]. *In vitro* studies demonstrated that stimulation with vitamin D lowered the activity of the wild type HTRA1 in a human retinal pigment epithelial cell line (ARPE-19) [48].

In conclusion, data from various observational studies indicate increased risk of early or advanced AMD in individuals with vitamin D deficiency. On the other hand, there are experimental evidences suggesting that vitamin D inhibits the inflammatory reaction and angiogenetic process involved in the development of AMD. Thus, it will be interesting to conduct randomised controlled clinical trials to assess the effects of vitamin D supplementation in the development or progression of AMD.

CHAPTER 5

VITAMIN D INSUFFICIENCY AND SUPPLEMENTATION

Vitamin D has captured the attention of the scientific and medical communities, regulatory agencies, the food industry, and the public over the past 15 years. This is evidenced by the explosion of scientific literature, a dramatic increase in physician-requested tests for patient vitamin D status in some countries, a number of authoritative re-evaluations of dietary recommendations, and sales of vitamin D supplements and the increased number of vitamin D-fortified food products coming on the market [1].

5.1 VITAMIN D DEFICIENCY, INSUFFICIENCY AND ADEQUACY

It is still discussed which vitamin D status is optimal for health and how to reach this target.

As regards minimal targets for vitamin D concentrations, there are currently two main categories of opinion [10]. The US Endocrine Society issued recommendations regarding vitamin D insufficiency in July 2011, which differ from those of the Institute of Medicine (IOM) on several points: they recommend a higher treatment target (≥ 75 nmol/L, i.e. 30 ng/mL) for health benefits; they state that individuals below 50 nmol/L (20 ng/mL) should be considered as vitamin D deficient [10]. When using these cutoffs, only 2.7% of American subjects would be 25(OH)D sufficient, 13.9% 25(OH)D insufficient, and 83.4% 25(OH)D deficient. In parallel, the IOM considers that the recommended daily allowance for vitamin D should lead to serum 25(OH)D levels of at least 50 nmol/L and that individuals below that level should receive vitamin D supplementation (Table 17). The IOM did not find that a level of serum 25(OH)D of > 75 nmol/L would be beneficial for bone health. They reported that the cutoff of 50 nmol/L to define deficiency in the general population is inconsistent with the data and exaggerates the number of vitamin D-deficient people. These recommendations are in line with those of the IOF (International Osteoporosis Foundation) and other bodies, such as the Standing Committee of European Doctors and the Swiss Federal Commission for Nutrition [10, 99, 100].

Table 17: Serum 25(OH)D concentration and health according to the IOM 2011

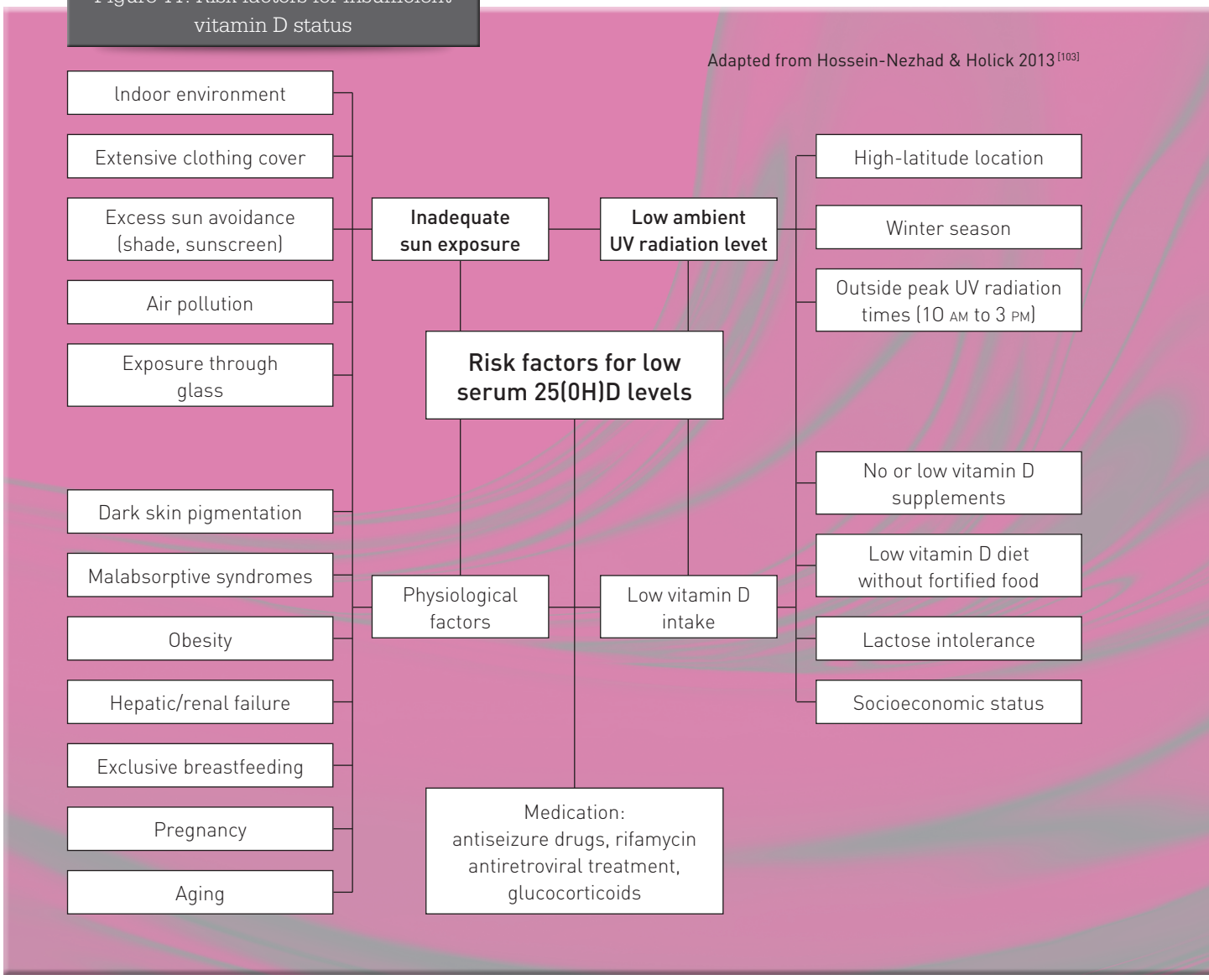
nmol/L	ng/mL*	Health status	Vitamin D status
<30	<12	Associated with vitamin D deficiency, leading to rickets in infants and children and osteomalacia in adults	Deficiency
30–50	12–20	Generally considered inadequate for bone and overall health in healthy individuals	Insufficiency
≥ 50	≥ 20	Generally considered adequate for bone and overall health in healthy individuals	Adequacy
> 125	> 50	Emerging evidence links potential adverse effects to such high levels, particularly > 150 nmol/L (> 60 ng/mL)	Toxicity

*1 nmol/L=0.4 ng/mL - Data from the National Institute of Health [101]

5.2 RISK FACTORS OF VITAMIN D DEFICIENCY

In general population, increasing age, female sex, non-white race, diabetes, current smoking, high BMI, are independently associated with an increased odds of being deficient in vitamin D [102]. Other risk factors includes a lack of exposure to sufficient sunlight, inadequate dietary intake and supplementation, medication use, sunscreen use, covering all skin with clothing, and skin pigmentation [6] (Figure 11).

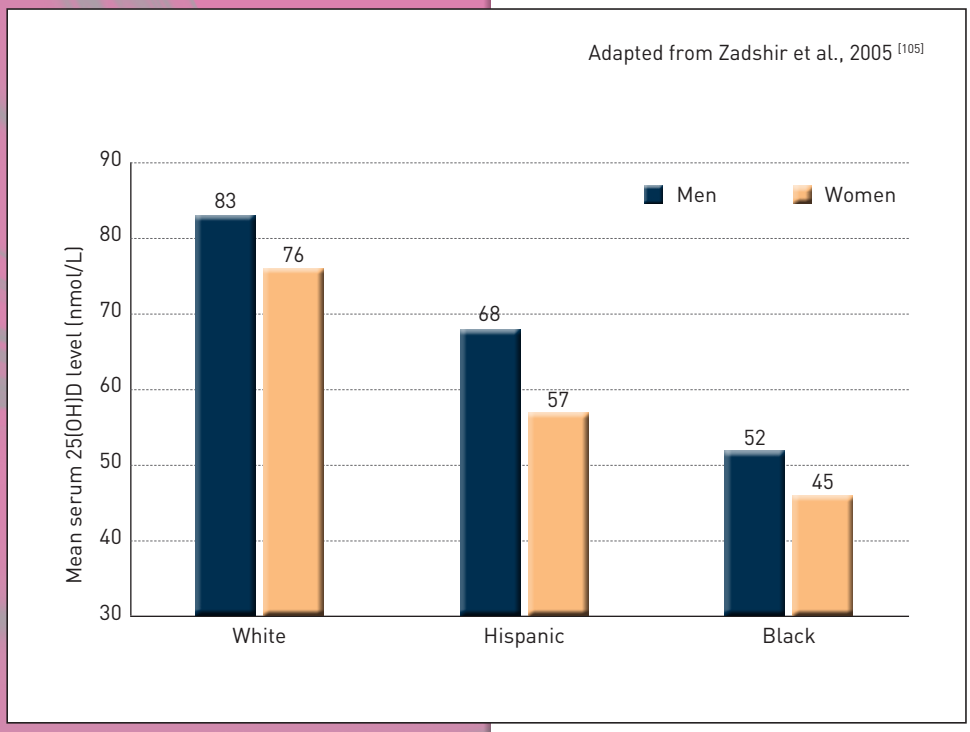
Figure 11: Risk factors for insufficient vitamin D status



More than 50% of the world's population is at risk for vitamin D deficiency [104].

In 2005, using data from the third National Health and Nutrition Examination Survey (NHANES III) including (15,390 adults ≥ 18 years of age), the mean serum levels of 25(OH)D were lower among female than male participants (71.1 vs 78.7 nmol/L; p=0.003) and among the elderly (≥ 65 years of age vs 40-59 and 18-39) than young participants. White men and women had higher mean levels of vitamin D (83.0 ng/mL and 76.0 nmol/L, respectively) than Hispanic men and women (68.3 and 56.7 nmol/L, p<0.0001) and Black men and women (52.2 nmol/L and 45.3 nmol/L, p<0.0001). The prevalence of both mild-moderate and severe deficiency of vitamin D was higher among women (p<0.0001) and minority populations (p<0.0001). However, even among White men, 34% had low vitamin D levels in US adults (Figure 12)[105].

Figure 12: Vitamin D status according to gender in the US population



The problem of vitamin D deficiency is not only prevalent among the elderly but more generally to women, dark-skin and obese people.

Elderly individuals are at risk for vitamin D deficiency because of poor dietary vitamin D intake, reduce mobility, decreased exposure to sunlight (because of reduced outdoor activity) and decline in renal function [106]. Capacity of skin to produce vitamin D is also decreased in the elderly, since ageing results in decreased amount of cutaneous 7-dehydrocholesterol, and by 70 years of age vitamin D3 synthesis is reduced approximately by 75% [3]. In addition, poor dietary intake of vitamin D in the elderly was shown in the POLA study (French Mediterranean area study). The highest risk of clinical deficiency was related to vitamin D, with only 1% of subjects out of risk [107] (i.e. 99% of subjects > 70 years had dietary vitamin D intake lower than 5 μg/day).

In the ALIENOR study, 27% of elderly subjects were deficient (<25nmol/L) and 56% were insufficient (25-49 nmol/L) [80]. In elderly subjects ≥65 years in general population in Spain, severe deficiency (< 15 ng/mL) was shown in 35% of subjects and insufficiency (< 30 ng/mL) in 86% [108].

Women are generally more prone to low 25(OH)D concentrations than men (possibly because of a positive correlation of 25(OH) vitamin D with testosterone levels). More than one-half of postmenopausal women have a 25(OH)D level <30 ng/mL [106].

Obese individuals require 2-5 times more vitamin D to treat and prevent vitamin D deficiency because fat can sequester vitamin D [109]. These differences may be due in part to lower levels of exercise and sunlight exposure in obese persons than lean persons [15].

5.3 VITAMIN D STATUS IN EUROPE

Knowledge of the distributions of serum 25(OH)D concentrations in representative populations, with appropriate consideration of sex, life stage, ethnicity, and seasonality, is critical for the quantification of vitamin D deficiency as well as for devising effective strategies for its prevention [1]. A recent systematic review of vitamin D status in populations worldwide clearly showed that the variability in mean serum 25(OH)D concentrations across European countries was large, and even within a country, the variability from different studies ranged from 10% to 300% [1, 110]. The International Osteoporosis Foundation and DSM Nutritional Products (DSM) have recently developed a map to provide a worldwide representation of vitamin D status. The map is based on a systematic review of worldwide literature published between 1990 and 2011. This review confirmed a north-south gradient in Europe, with Scandinavian countries generally showing higher values for circulating 25(OH)D than in Southern Europe (Table 18). The somewhat unexpected north-south gradient, with a positive correlation between vitamin D status and latitude may be influenced by diets containing more oil-rich fish, a higher use of cod liver oil and other vitamin D supplementation, and population differences in skin pigmentation [110].

Table 18: Vitamin D status in adults in European countries

Country	Sample size	Age (Years)	Gender	Vitamin D status 25(OH)D (nmol/L)
Austria	1048	21-76	M, W	52.2 (winter)
Belgium	542	≥ 20	M,W	71.4 (M), 73.4 (W)
Czech Republic	47	62.3	W	58.2
Denmark	125	35-65	M, W	25.5
Finland	4097	40-69	M, W	43.6
France	1569	35-65	M, W	61.0
Germany	4030	18-79	M	45.2 (M), 44.7 (W)
Italy	697	69-80	W	37.9 (winter)
Netherland	1319	65-88	M, W	53.2
Poland	274	60-90	W	33.5 (winter)
Spain	237	65-93	M, W	42.9 (winter)
Sweden	986	75	W	95.0
Switzerland	3276	25-74	M, W	50.0
UK	924	≥ 65	M, W	49.7

M: Men, W: women - Ref: Adapted from Spiro & Buttries, 2014 [111]

40% of adults in European Union have insufficient status in vitamin D and 13% are deficient in vitamin D [1].

The prevalence of vitamin D deficiency (defined as serum 25(OH)D < 30 nmol/L) based on standardised 25(OH)D data has been recently re-estimated based on a metaanalysis of 55844 individuals of 18 nutritional and health surveys from various European Union countries [1]. An overall pooled estimate, irrespective of age group, ethnic mix, and latitude of study populations, showed that 13.0% of the European individuals had serum 25(OH)D concentrations <30 nmol/L on average in the year, with 17.7% and 8.3% in those sampled during the extended winter (October-March) and summer (April-November) periods, respectively. According to an alternate suggested definition of vitamin D deficiency (<50 nmol/L), the prevalence was 40.4%. Dark-skinned ethnic subgroups had much higher (3- to 71-fold) prevalence of serum 25(OH)D <30 nmol/L than did white populations.

5.4 INTEREST OF VITAMIN D SUPPLEMENTATION

Vitamin D deficiency has been described as being pandemic, with associated direct and indirect cost for Europe estimated to be running at hundreds of billion Euro [1]. Data from the NHANES III in adults ≥ 20 years of age, showed that vitamin D deficiency was associated with a 26% higher risk of all-cause mortality, independent of baseline demographics, traditional and non-traditional CVD risk factors, and measures of a healthy lifestyle [102]. Given that a major part of the general population had vitamin D serum concentrations lower than the recommended levels, vitamin D supplementation of deficient subjects is a major public health concern.

Vitamin D deficiency is in part due to the inadequate fortification of foods with vitamin D and the misconception that a healthy diet contains an adequate amount of vitamin D [104].

SUN EXPOSURE

A simple way to obtain vitamin D is from moderate exposure to sunlight [112]. In some regions, exposure of arms and legs for 5 to 30 minutes between the hours of 10 am and 3 pm twice a week can be adequate to prevent vitamin D deficiency [3]. Studies conducted in healthy volunteers have shown that exposure of the whole body for 3 weeks is equivalent to a daily intake of 10 000 IU (250 μg) over the same time period, i.e. a quantity sufficient to cover vitamin D requirements for almost 6 months. However, in most European countries, i.e. in latitude above 35° North, there is no cutaneous vitamin D3 synthesis during the winter months [113]. Whole body irradiation with effective UV light has resulted in plasma 25(OH)D levels comparable to those observed during oral consumption of 250 μg of vitamin D daily [12]. However, tanning beds provide variable levels of UVA and UVB rays and therefore may be not a reliable source of vitamin D [33]. In addition, it is well documented that excessive exposure to sunlight, especially the number of sun burning experiences, is related to increased risk of squamous and basal cell carcinoma [6].

FOOD FORTIFICATION WITH VITAMIN D

Vitamin D intakes are lower than dietary recommendations in most populations [114]. Fish consumption increases vitamin D concentrations in healthy adult, but could not fulfil the recent recommendations for the daily dietary amount of vitamin D as shown in a recent meta-analysis of randomised controlled trials [115]. Food fortification with vitamin D is a reasonable approach to satisfying the body's requirement in vitamin D and one preventive strategy to reach the entire population. In several countries, various foods are now fortified in vitamin D including milk and margarine, but also orange juice, cereals, and oily fish [112, 114]. Nonetheless, this fortification practice does not seem optimal for improving vitamin D status in the general population [114].

VITAMIN D SUPPLEMENTS

Vitamin D supplements are widely available and relatively inexpensive [6]. Alternatively multivitamin containing 400 IU vitamin D, and a vitamin D supplement containing 400 or 1000 IU vitamin D may be appropriate [112].

It has been suggested that whereas vitamin D2 and vitamin D3 may equally increase 25(OH)D concentrations when supplemented daily, vitamin D3 may raise 25(OH)D concentrations more than vitamin D2 if single or infrequent bolus doses are administered [113].

Owing to its slow turnover in the body (half-life of about two months, vitamin D is often administered weekly in equivalent doses instead of daily. Depending on the dose and the duration of supplementation, resulting 25(OH)D concentrations may be comparable or somewhat lower with weekly compared to daily supplementation, respectively [113].

TOLERABLE UPPER LEVEL INTAKE

Following a request of the European Commission in 2012, the tolerable upper intake level (UL) of vitamin D has been revised by an European Food Safety Authority (EFSA) panel [113]. Hypercalcaemia was selected as the indicator of toxicity. Hypercalcaemia is associated with various symptoms (Table 19). The primary consequences of sustained hypercalcaemia are nephrolithiasis (kidney stones), nephrocalcinosis, and a decrease in kidney function.

It was agreed that when very large doses of vitamin D is ingested, the concentration of 25(OH)D in serum increases, but that of the active metabolite $1,25(OH)_2D$ is unchanged or even reduced. Very high serum 25(OH)D concentrations may lead to hypercalcaemia, which is considered the critical effect of excess intake of vitamin D. Hypercalciuria can be associated with hypercalcaemia, but it can also occur without.

The tolerable upper intake level was defined as the levels of ingested vitamin D that are unlikely to pose a risk of adverse health effects for all relevant population groups. It has been established at 100 µg/day in adults, including pregnant and lactating women. For children and adolescents, the UL was set at 50 µg/day for ages 1-10 years, and at 100 µg/day for ages 11-17 years. For infants up to one year of age, the UL is 25 µg/day (Table 20).

Table 19: Clinical symptoms associated with hypercalcaemia [113]

• Fatigue	• Tachycardic arrhythmia
• Muscular weakness	• Soft tissue calcification
• Anorexia	• Failure to thrive
• Nausea	• Weight loss
• Vomiting	• Hypercalciuria
• Constipation	

Table 20: Tolerable upper intake level for vitamin D according to the EFSA panel [113]

Age (years)	Tolerable upper intake level for vitamin D	
	μg/day	IU/day
0-1	25	1000
1-10	50	2000
11-17	100	4000
Adults (≥ 18)*	100	4000

* Including pregnant and lactating women

5.5 CURRENT CLINICAL RECOMMENDATIONS WORLDWIDE

INSTITUTE OF MEDICINE (IOM)

In 2011, the IOM published new dietary reference intakes (DRIs) intended to serve as a guide for good nutrition and provide the basis for the development of nutrient guidelines in both the United States and Canada. The committee assumed minimal sun exposure when establishing the DRIs for vitamin D. The IOM considered that Recommended Dietary Allowances (RDAs) of 600 IU/day for ages 1-70 years (including pregnant and lactating women) and 800 IU/day for ages 71 years and older, corresponding to a serum 25(OH)D level of at least 20 ng/ml (50 nmol/L), meet the requirements of at least 97.5% of the population [116].

The IOM was concerned about the possibility of adverse consequences from over-supplementation. Thus, the DRI process also specifies the tolerable upper intake level (UL; the highest daily intake of the nutrient that is likely to pose no risk) (see Table 21). The indicators considered in the determination of ULs included emerging evidence of a U-shaped relationship for all-cause mortality, cardiovascular disease, selected cancers, falls and fractures [116].

Table 21: The IOM Recommended Dietary Allowances for vitamin D

Age	RDA (IU/day)	Serum 25(OH)D	UL
0–6 months	400 IU* (10 µg)	50 nmol/L (20 ng/mL)	1000 IU/d
6–12 months	400 IU* (10 µg)	50 nmol/L (20 ng/mL)	1500 IU/d
1–70 years	600 IU (15 µg)	50 nmol/L (20 ng/mL)	2500 - 4000 IU/d
> 70 years	800 IU (20 µg)	50 nmol/L (20 ng/mL)	4000 IU/d

* RDA (Recommended Dietary Allowance): average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%-98%) healthy people; UL: level above which there is risk of adverse events. Adapted from Ross et al., 2011 [116].
Reflects AI reference value rather than RDA. RDAs have not been established for infants.

ENDOCRINE SOCIETY

In 2010, the US Endocrine Society provides guidelines to clinicians for the evaluation, treatment, and prevention of vitamin D deficiency with an emphasis on the care of patients who are at risk for deficiency as defined by 25(OH)D < 20 ng/mL (50 nmol/L) [99].

They consider that adults aged 19-50 years require at least 600 IU/day of vitamin D to maximise bone health and muscle function. It is unknown whether 600 IU/day is enough to provide all the potential nonskeletal health benefits associated with vitamin D. However, to raise the blood level of 25(OH)D consistently above 30 ng/mL may require at least 1500-2000 IU/day of vitamin D.

In adults aged 50-70 and > 70 years, they suggest at least 600 and 800 IU/day, respectively, of vitamin D. Whether 600 and 800 IU/day of vitamin D are enough to provide all of the potential nonskeletal health benefits associated with vitamin D is not known at this time.

Table 22: US Endocrine Society recommendations [99]

Age	Daily requirement	UL
1–12 months	400-1000 IU (10 -25 µg)	2000 IU/d
1–18 years	600-1000 IU (15-25 µg)	4000 IU/d
> 18 years	1500-2000 IU (32.5-50 µg)	10 000 IU/d

UL: level above which there is risk of adverse events

US PREVENTIVE SERVICES TASK FORCE (USPSTF)

In 2013, the US Preventive Services Task Force (USPSTF) made recommendations based on the premise that vitamin D deficiencies are common among all age groups. The Society prefers 30 ng/mL of 25(OH)D as the target level for maximum benefits. By that criterion, virtually all US, Canadian, and European adults are deficient in vitamin D and require daily supplements of 1500 to 2000 IU. For adults with demonstrated deficiency, the Society recommends treatment with 50 000 IU of vitamin D once a week or daily supplementation of 6000 IU for 8 weeks, followed by 1500 to 2000 IU for maintenance [118].

RECOMMENDATION OF THE NICE

For the NICE, in 2014, the current reference nutrient intakes for vitamin D i.e. the amount of a nutrient needed to meet 97.5% of individual are 8.5 μg/day (340 IU/day) for infants up to 6 months, 7 μg/day (280 IU/day) for children between 6 months and 3 years, and 10 μg/day (400 IU/day) for women during pregnancy and lactation, and for adults over 65 years old. There is currently no recommendation for people aged between 4 and 65 years. It is assumed that the action of sunlight on skin will provide adequate vitamin D, except for specific at-risk groups, such as women whose clothing conceals them fully or those confined indoors. At-risk groups are currently advised to take a supplement that meets 100% of the reference nutrient intake i.e. 10 μg/day (400 IU/day) [117].

CONCLUSION

- ***Vitamin D is well known to be good for bone health and other pathologies.***
- ***Recent data show that vitamin D has an impact on the retina, age-related macular degeneration and diabetic retinopathy.***
- ***Vitamin D can act on AMD thanks to inhibition of inflammation and angiogenesis.***
- ***Vitamin D deficiency is highly prevalent particularly in the elderly so it's important to supplement.***

Vitamin D plays an essential role in bone health and other diseases including immune/inflammatory diseases, cancer, diabetes, and brain diseases. In various tissues, the active form of vitamin D binds to a specific receptor and controls the activation or repression of gene transcription. Overall, vitamin D is a potential modulator of cell proliferation, differentiation and apoptosis.

Observational studies consistently showed an inverse relationship between vitamin D status and retinal diseases, including diabetic retinopathy and age-related macular degeneration. High vitamin D status may protect against early, and advanced AMD including neovascular AMD. This was recently confirmed in genetic and nutritional studies suggesting that high intake of vitamin D was associated with less severe AMD in homozygotic twins. Low intakes of vitamin D have been also significantly associated with neovascular AMD. Altogether, observational studies consistently suggest a cause and effect relationship between vitamin D status and AMD. The causal role of vitamin D in AMD physiopathology is supported by experimental studies which demonstrate potent inhibitory activity of vitamin D on retinal inflammation and angiogenesis.

Clinical practice guidelines recommend nutritional vitamin D supplements for vitamin D insufficiency and deficiency. Although, the optimal vitamin D status for health is still a matter of debate, around 40% of individuals in the general population in Europe have insufficient serum level of vitamin D according to cut-offs defined by scientific societies. Specifically, the level of vitamin D adequacy in retinal disease remains to be determined in interventional clinical studies. Other randomised clinical trial should be carried out to confirm the interest of vitamin D supplementation in diabetic retinopathy or AMD. In the meantime, vitamin D supplements are relatively inexpensive and generally well tolerated and since there is currently no treatment to prevent the development of retinopathies, vitamin D supplementation may be an interesting care option.

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