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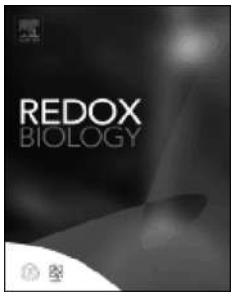

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Carsten Carlberg, Marianna Raczyk, Natalia Zawrotna

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Vitamin D: a master example of nutrigenomics

Carsten Carlberg[1,2#], Marianna Raczyk[1] and Natalia Zawrotna[1]

[1]*Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, PL-10748 Olsztyn, Poland*

[2]*School of Medicine, Institute of Biomedicine, University of Eastern Finland, FI-70211 Kuopio, Finland*

[#]**Corresponding author**: Prof. Carsten Carlberg
Institute of Animal Reproduction and Food Research
Polish Academy of Sciences
ul. Juliana Tuwima 10
PL-10748 Olsztyn
Poland
Tel.: +48-89-523-4612
E-mail: c.carlberg@pan.olsztyn.pl

Abstract

Nutrigenomics attempts to characterize and integrate the relation between dietary molecules and gene expression on a genome-wide level. One of the biologically active nutritional compounds is vitamin D_3, which activates *via* its metabolite 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) the nuclear receptor VDR (vitamin D receptor). Vitamin D_3 can be synthesized endogenously in our skin, but since we spend long times indoors and often live at higher latitudes where for many winter months UV-B radiation is too low, it became a true vitamin. The ligand-inducible transcription factor VDR is expressed in the majority of human tissues and cell types, where it modulates the epigenome at thousands of genomic sites. In a tissue-specific fashion this results in the up- and downregulation of primary vitamin D target genes, some of which are involved in attenuating oxidative stress. Vitamin D affects a wide range of physiological functions including the control of metabolism, bone formation and immunity. In this review, we will discuss how the epigenome- and transcriptome-wide effects of $1,25(OH)_2D_3$ and its receptor VDR serve as a master example in nutrigenomics. In this context, we will outline the basis of a mechanistic understanding for personalized nutrition with vitamin D_3.

Keywords

Vitamin D, VDR, vitamin D target genes, epigenome, transcriptome, immune system, personalized nutrition.

Abbreviations

$1,25(OH)_2D_3$	1α,25-dihydroxyvitamin D_3
$25(OH)D_3$	25-hydroxyvitamin D_3
ACVRL1	activin A receptor like type 1
ATAC-seq	assay for transposase-accessible chromatin using sequencing
BRD7	bromodomain containing 7
CALB1	calbindin 1
CAMP	cathelicidin antimicrobial peptide
CAR	constitutive androstane receptor
CCN	cyclin
CD	cluster of differentiation
CDKN	cyclin dependent kinase inhibitor
CEBP	CCAAT enhancer binding protein
ChIP-seq	chromatin immunoprecipitation sequencing
CTCF	CCCTC binding factor
CXCL	C-X-C motif chemokine ligand
CYP	cytochrome P450
DHCR7	7-dehydrocholesterol reductase
DNMT	DNA methyltransferase
EREG	epiregulin
ESR	estrogen receptor
FAIRE-seq	formaldehyde-assisted identification of regulatory elements followed by sequencing
FBP1	fructose-bisphosphatase 1
FGF23	fibroblast growth factor 23
FN1	fibronectin 1
FOS	Fos proto-oncogene, AP-1 transcription factor subunit
FXR	farnesoid X receptor
G0S2	G0/G1 switch 2

GABPα	GA binding protein transcription factor α
GC	GC vitamin D binding protein
GR	glucocorticoid receptor
GTEx	Genotype-Tissue Expression
HAT	histone acetyltransferase
HBEGF	heparin binding EGF like growth factor
HDAC	histone deacetylase
HLA	human leukocyte antigen
IGF1	insulin-like growth factor 1
IL	interleukin
INSR	insulin receptor
JUN	Jun proto-oncogene, AP-1 transcription factor subunit
KDM	lysine demethylase
KMT	lysine methyltransferase
LILRB4	leukocyte immunoglobulin like receptor B4
LMNA	lamin A/C
LRRC25	leucine rich repeat containing 25
LXR	liver X receptor
MAPK13	mitogen-activated protein kinase 13
MHC	major histocompatibility complex
MYC	MYC proto-oncogene, BHLH transcription factor
NAD	nicotinamide adenine dinucleotide
NFE2L2	NFE2 like BZIP transcription factor 2, also called NRF2
NGS	next-generation sequencing
NINJ1	ninjurin 1
NK	natural killer
PARM1	prostate androgen-regulated mucin-like protein 1
PBMC	peripheral blood mononuclear cell
PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4

Pol II	RNA polymerase II
PTH	parathyroid hormone
PXR	pregnane X receptor
RNA-seq	RNA-sequencing
ROS	reactive oxidative species
RXR	retinoid X receptor
SEMA6B	semaphorin 6B
SPI1	spleen focus forming virus proviral integration oncogene, also called PU.1
SRGN	serglycin
STAB1	stabilin 1
TAD	topologically associated domain
TET	ten-eleven translocation
T_H	T helper
THBD	thrombomodulin
THEMIS2	thymocyte selection associated family member 2
T_{reg}	T regulatory
TREM1	triggering receptor expressed on myeloid cells 1
TRPV6	transient receptor potential cation channel subfamily V member 6
TSS	transcription start site
UV	ultraviolet
VDR	vitamin D receptor
VKORC1	vitamin K epoxide reductase complex subunit 1

Introduction

Nutritional genomics, also referred to as nutrigenomics, describes the relation between what we eat and how our genome reacts to this environmental trigger[1]. Nutrigenomics developed as a discipline for the epigenome- and transcriptome-wide description of the effects of diet in health and disease. Various next-generation sequencing (NGS) methods, such as ATAC-seq (assay for transposase-accessible chromatin using sequencing), FAIRE-seq (formaldehyde-assisted identification of regulatory elements followed by sequencing), ChIP-seq (chromatin immunoprecipitation sequencing) and RNA-seq (RNA-sequencing), are based on the knowledge of the complete sequence of genome, *i.e.*, during the past 20 years these unbiased approaches for the assessment of the effects of dietary molecules on the epigenome and transcriptome could be developed[2]. In addition, nutrigenomics uses proteomic and metabolomic methods, such as mass spectroscopy, that are independent from NGS technologies, but at present they do not allow to detect the completeness of proteins and metabolites present in an investigated cell type or tissue[3]. Importantly, most nutrigenomic approaches integrate data from different omics levels being obtained by *in vitro* cell culture, model organisms and human intervention studies[4].

Molecules derived from our daily diet represent the major environmental influence, to which we are voluntarily exposed to. Many of these macro- and micronutrients, such as lipids and lipophilic vitamins, do not act only as a storage of energy, but have intra- and intercellular signaling properties that control a number of physiological processes, such as cellular metabolism and growth. A key aspect of nutrigenomics is to describe and mechanistically understand the signaling pathways of nutritional molecules. Since diet is a complex mixture of hundreds to thousands of biologically active compounds, the primary focus is taken often on individual molecules. Some of these compounds have a direct effect on gene expression, while others need to be first metabolized, in order to modulate the activity of transcription factors or chromatin modifying enzymes[5]. Examples are secondary metabolites like genistein from green tea, resveratrol from red grapes and curcumin from curcuma[6]. Another interesting example is the micronutrient vitamin D_3 that we can take up from certain diets, such as fatty fish, but also produce endogenously, when we expose our skin to sufficient doses of ultraviolet (UV)-B radiation[7]. Importantly, when vitamin D_3 is metabolized into $1,25(OH)_2D_3$, it acts as a high affinity ligand for the transcription factor VDR, *i.e.*, it has direct epigenome- and transcriptome-wide effects[8].

The key physiological functions of $1,25(OH)_2D_3$ are the regulation of calcium homeostasis, which is essential for bone mineralization, and the modulation of the immune system by stimulating innate immunity and preventing overreactions of adaptive immunity[9,10]. In addition to these major, mechanistically well understood physiological function, vitamin D was

reported to be involved in numerous other processes in health and disease. For example, vitamin D is suggested to delay cellular senescence *via* the reduction of oxidative stress[11].

For micronutrients like vitamin D_3 often the question is raised, whether their serum levels are sufficient for obtaining maximal health benefits for the individual. A related question is, if there are interindividual variations in the need for the micronutrient, *i.e.*, whether there is a need for personalized supplementation. Accordingly, this review will not only discuss nutrigenomics of vitamin D_3 on the level of the compound's mechanistic function as a regulator of gene expression but will also address the personalized responses to the micronutrient in physiological settings like responses of the immune system.

Vitamin D and its metabolites

In keratinocytes of human skin, a reaction takes place that converts 7-dehydrocholesterol, which is a direct precursor of cholesterol, into pre-vitamin D_3 (**Fig. 1, left**). The latter is a thermodynamically unstable molecule that rapidly isomerizes into vitamin D_3[12]. This reaction is non-enzymatic but requires energy provided by UV-B (290-315 nm) radiation. Interestingly, at excessive UV-B exposure pre-vitamin D_3 can transform into the compounds tachysterol and lumisterol, in order not to produce too high amounts of vitamin D_3[13].

All cholesterol-producing species are able to synthesize vitamin D_3, when they are exposed to sunlight of sufficient intensity. However, species living in a cholesterol-rich environment, such as blow flies and tapeworms, gave up energy and oxygen consuming cholesterol synthesis[14]. Interestingly, also UV-B-radiated plants and mushrooms produce a vitamin D isomer, but since they use the sterol ergosterol as a precursor, the outcome is vitamin D_2[15] (**Fig. 1, right**). In contrast to vitamins C and E, vitamin D has no scavenging function for reactive oxidative species and other free radicals. However, the absorption of UV-B by 7-dehydrocholesterol functions as a shield against radiation damage in animals and plants. Therefore, even simple eukaryotes, such as phytoplankton, synthesize vitamin D_3 as a side product of a sun-shielding effect but they do not use vitamin D_3 for any endocrine function[16]. Interestingly, vitamin D_3 production in phytoplankton is the main reason why the molecules accumulate in the marine food chain[17].

Both vitamin D_3 and vitamin D_2 are biologically inert secosteroids with an open B-ring in their sterol backbone that differ only in their side chain. In human intestine, vitamin D_3 is taken up more effectively[18] but both vitamin D isomers are used for supplementation and food fortification[19]. In the bloodstream both compounds (as well as their metabolites) are bound to the serum glycoprotein GC (GC vitamin D binding protein) and transported from keratinocytes (when endogenously produced) or enterocytes (when taken up by diet) to the liver[20]. The enzymes CYP2R1 (cytochrome P450 family 2 subfamily R member 1) in microsomes and

CYP27A1 in mitochondria hydroxylate both vitamin D_3 and vitamin D_2 at C-25 leading to the pre-hormones 25-hydroxyvitamin D_3 ($25(OH)D_3$) and $25(OH)D_2$[21] (**Fig. 1, bottom**). In proximal tubule cells of the kidneys, the enzyme CYP27B1 hydroxylates both metabolites at C-1, which creates the nuclear hormones $1,25(OH)_2D_3$ and $1,25(OH)_2D_2$, respectively[22,23], that bind already at a concentration of 0.1 nM to the nuclear receptor VDR[24]. In addition to $1,25(OH)_2D_3$ production in the kidneys, cells of the innate immune system like dendritic cells, macrophages and monocytes, keratinocytes and osteoblasts express the *CYP27B1* gene and can synthesize $1,25(OH)_2D_3$ for autocrine and paracrine purposes[25].

Since the metabolite $25(OH)D_3$ (**Fig. 1**) is with a serum half-life of more than 14 days the metabolically most stable and abundant vitamin D compound[26], it is used as a biomarker indicating the individual's vitamin D status[27]. Serum concentrations of less than 50 nM $25(OH)D_3$ (20 ng/ml) are considered as insufficient[28], because they significantly increase the risk for musculoskeletal disorders in children (rickets) and adults (osteomalacia and fractures)[29]. Furthermore, vitamin D insufficiency contributes to a number of immunological disorders, such as multiple sclerosis[30,31], rheumatoid arthritis[32], inflammatory bowel disease[33], type I diabetes[34], and is associated with severe consequences from infections with the intracellular bacterium *mycobacterium tuberculosis*[35,36], influenza virus or severe acute respiratory syndrome coronavirus type 2[37,38]. In order to obtain a clinical benefit from these non-skeletal effects of vitamin D, the vitamin D status should be in the range of 75-100 nM (30-40 ng/ml) $25(OH)D_3$[39].

During winter times in the Northern hemisphere, there is above a latitude of 38°N a period of 1-5 months, in which the UV-B component of sunlight reaching the surface is too low for vitamin D_3 synthesis ("vitamin D winter"). Therefore, the migration of our species out of Africa as well as modern lifestyle characterized by predominant indoor activities[40] made vitamin D_3 a micronutrient that needs to be obtained by diet or supplemented by pills. Since average human diet does not contain much fatty fish (the main source of vitamin D_3 in diet[41]) or UV-B-irradiated mushrooms[42], it is low in vitamin D_3 or vitamin D_2. In order to prevent vitamin D deficiency, it is recommended to take at least 25 µg (1,000 IU) vitamin D_3 per winter day[43], but daily doses of up to 100 µg are considered to have a positive effect on health. However, caution needs to be taken, since long-term overdosing with vitamin D_3 or its metabolites can cause hypercalcemia and tissue calcification[44].

In summary, vitamin D_3 can be synthesized endogenously in UV-B exposed skin, but due to human migration and lifestyle changes it became a physiologically important micronutrient that needs to be taken up *via* diet or directly supplemented. A sufficient vitamin D status is essential for the health of our bones and immune system[28].

Physiological role of vitamin D and VDR

VDR is one of some 1,600 transcription factors encoded by our genome, but stands out from this large family of regulatory proteins by being directly modulated in its activity by a small lipophilic molecule like $1,25(OH)_2D_3$. This property makes VDR very comparable to the receptors ESR (estrogen receptor) and GR (glucocorticoid receptor) that have large medical impact, because they are activated by the female sex steroid estrogen and the stress hormone cortisol, respectively. All together there are only 13 classical endocrine members within the superfamily of nuclear receptors. Interestingly, VDR's closest relatives within the superfamily are the adopted orphan receptors PXR (pregnane X receptor), CAR (constitutive androstane receptor), FXR (farnesoid X receptor) and LXR (liver X receptor) α and β[45]. All six nuclear receptors bind and get activated by moderate levels of the cholesterol derivatives bile acids and/or oxysterols[46-49]. However, only VDR learned some 550 million years ago to accommodate with high affinity $1,25(OH)_2D_3$[50].

The receptors FXR and LXRs are well known for the regulation of lipid metabolism pathways, while PXR and CAR control more specifically xenobiotic detoxification pathways. This suggests that very likely the evolutionary first role of VDR was the regulation of metabolic pathways, such as those controlled by CYP enzymes[51]. It is likely that in this context VDR and its ligand got an impact on attenuating oxidative stress, *e.g.*, by modulating the expression of the *NFE2L2* (NFE2 like BZIP transcription factor 2) gene, the encoded protein of which is often referred to as NRF2[11].

One of the most prominently responding vitamin D target genes is *CYP24A1*, which encodes for an enzyme that initiates the degradation of $1,25(OH)_2D_3$ and $25(OH)D_3$. Moreover, investigating *CYP24A1* gene regulation provides molecular insight into the coordinated mechanistic actions of $1,25(OH)_2D_3$ in the kidney that regulate mineral homeostasis[52]. In contrast, *CYP27B1* encodes for an enzyme that is essential for $1,25(OH)_2D_3$ production, while *CYP19A1* is the gene of the aromatase enzyme catalyzing the last step in estrogen biosynthesis. Both genes are downregulated vitamin D targets. Other important vitamin D target genes with metabolic function are *FBP1* (fructose-bisphosphatase 1)[53] and *PFKFB4* (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4)[54], which encode for enzymes with key functions in gluconeogenesis.

VDR became an important regulator of immunity, since both innate and adaptive cells need substantial amounts of energy for their differentiation and proliferation[55]. For example, inducing tolerogenic properties to dendritic cells requires the reprogramming of their glucose metabolism *via* the upregulation of *PFKFB4*. Vitamin D supports monocytes and macrophages in their fight against tuberculosis[56] through increasing the levels of the anti-microbial peptide CAMP (cathelicidin antimicrobial peptide)[57] or the plasma membrane-anchored glycoprotein CD14 (cluster of differentiation 14) that functions as co-receptor for Toll-like receptors[58]. In

parallel, vitamin D prevents that cells of the adaptive immune system overreact. This involves the reduction of T_H (T helper) 1 cell counts and the increase of T_{reg} (T regulatory) and T_H2 cells[59,60]. Most cell types of the immune system show a fast turnover, in order to quickly respond to environmental changes[61]. For example, macrophages coordinate pathways of inflammation, metabolism and general stress response *via* vitamin D-triggered changes of their epigenome and transcriptome. Vitamin D stimulation causes first an increase and later the resolution of inflammation[62]. Mechanistically, this is mediated by a shift of M1 into M2 macrophages[63,64].

Epigenomic programming through VDR also happens during hematopoiesis, where the receptor acts together with the pioneer transcription factors SPI1 (spleen focus forming virus proviral integration oncogene) and CEBP (CCAAT enhancer binding protein) α as key regulators of myeloid differentiation[65]. Vitamin D affects the growth of hematopoietic stem cells[66] by regulating a family of *CXCL* (C-X-C motif chemokine ligand) genes, which are all located in gene cluster on chromosome 4. This cluster contains the up-regulated genes *CXCL1*, *CXCL5*, *CXCL7*, *CXCL8* and *EREG* (epiregulin) and the down-regulated genes *CXCL9*, *CXCL10* and *PARM1* (prostate androgen-regulated mucin-like protein 1)[9]. Further examples of immune related vitamin D target genes are *ACVRL1* (activin A receptor like type 1), *CD93*, *CEBPB*, *MAPK13* (mitogen-activated protein kinase 13), *FN1* (fibronectin 1), *NINJ1* (ninjurin 1), *LRRC25* (leucine rich repeat containing 25), *LILRB4* (leukocyte immunoglobulin like receptor B4), *SEMA6B* (semaphorin 6B), *THBD* (thrombomodulin), *SRGN* (serglycin), *TREM1* (triggering receptor expressed on myeloid cells 1) and *THEMIS2* (thymocyte selection associated family member 2) and, most of which encode for membrane proteins or secreted proteins[67]. In T cells VDR antagonizes the action of the transcription factors NFAT, AP1 and NFκB, so that the major growth factor for adaptive immune cells, the cytokine IL (interleukin) 2, is produced in lower amounts[68]. In dendritic cells, vitamin D inhibits their differentiation, maturation and the immuno-stimulatory capacity *via* the downregulation of the genes for the co-stimulatory molecules CD40, CD80 and CD86[69]. Finally, a real "hotspot" of vitamin D targets is the cluster of *HLA* (human leukocyte antigen) genes encoding for major histocompatibility complex (MHC) proteins of classes I and II[9]. In total, 10 of the 12 genes that encode for both chains of MHC class II receptors are downregulated by vitamin D[9]. This is a central mechanism how vitamin D reduces the risk for autoimmune diseases.

Since immune and transformed cells use the same pathways for controlling their growth[66,70], $1,25(OH)_2D_3$ and the VDR are able to inhibit cancer cell proliferation. Key vitamin D targets in cell cycle regulation are the upregulated tumor suppressor genes *CDKN1A* (cyclin dependent kinase inhibitor 1A) and *CDKN1B*, the cyclins *CCNC* (cyclin C), *CCND1*, and *G0S2* (G0/G1 switch 2) and the downregulated oncogenes *MYC* (MYC proto-oncogene, BHLH transcription factor), *JUN* (Jun proto-oncogene, AP-1 transcription factor subunit), *FOS* (Fos proto-oncogene, AP-1 transcription factor subunit), *JUND* and *JUNB*[71-78]. Thus, the anti-proliferative

effect of $1,25(OH)_2D_3$ and its synthetic analogues on cell lines of solid cancers and the differentiation-inducing effect on leukemia cell lines, which have been studied for 40 years[79,80], as well as the ability to induce apoptosis in many cell types, are related to vitamin D's function controlling the fate of immune cells[81]. Thus, the main effect of vitamin D against cancer is not inhibiting the growth of existing tumors but the stimulation of cytolytic T cells to detect and eliminate transformed cells already in an early stage[82].

In general, vitamin D is best known for regulating calcium homeostasis, which is essential for bone metabolism[83]. Accordingly, *PTH* (parathyroid hormone), *FGF23* (fibroblast growth factor 23), *CALB1* (calbindin 1) and *TRPV6* (transient receptor potential cation channel subfamily V member 6) are key vitamin D targets genes encoding for proteins with impact on calcium metabolism and bone turnover[84]. In the kidneys, there is an interesting regulatory network between $1,25(OH)_2D_3$, PTH and FGF23, in which vitamin D downregulates PTH but upregulates FGF23, while both PTH and FGF23 inhibit $1,25(OH)_2D_3$ synthesis by downregulating *CYP27B1* gene expression[85,86]. Since $1,25(OH)_2D_3$ is primarily synthesized in the kidney, PTH is produced in the parathyroid gland and FGF23 in bone, in this metabolically important regulatory network vitamin D cannot be replaced by other regulatory molecules. This explains why vitamin D deficiency has primarily a bone dysfunction phenotype[87]. Moreover, the regulatory network is even further extended by the finding that insulin and IGF1 (insulin-like growth factor 1) downregulate FGF23 production[88]. This is further complicated by the observation that the *INSR* (insulin receptor) gene is upregulated by $1,25(OH)_2D_3$[89]. Thus, vitamin D signaling has several connections with insulin signaling and may provide a hint how vitamin D deficiency may increase the risk for type 2 diabetes and the metabolic syndrome[90,91].

An alternative approach to judge the physiological impact of VDR and its ligand $1,25(OH)_2D_3$ is to compare the expression of the *VDR* gene in various human tissues and cell types. At present, the best source of such data is the big biology project GTEx (Genotype-Tissue Expression, https://gtexportal.org), through which gene expression data from 54 tissues obtained from 948 post-mortem donors are available[92] (**Fig. 2**). Interestingly, highest *VDR* expression is found in tissues of vitamin D_3 production (skin) and resorption (small intestine), while lowest levels of *VDR* mRNA is found in different regions of the brain. Between these extremes basically all investigated tissues show intermediate *VDR* expression. These data suggest that $1,25(OH)_2D_3$ should have an impact on the physiology of most human tissues and cell types.

Taken together, the transcription factor VDR is the only high affinity target of $1,25(OH)_2D_3$. This suggests that the functional profile of VDR and vitamin D are nearly identical showing pleiotropic actions related to metabolism, in particular calcium homeostasis, as well as immunity, cellular growth and differentiation. Interestingly, anti-cancer actions of vitamin D are based on the same mechanisms and genes that control immune cells.

Impact of the epigenome

Chromatin, the three-dimensional complex of genomic DNA and nucleosome-forming histones, is the physical expression of epigenetics[93,94]. The location of regulatory regions of a gene within loosely packed chromatin (euchromatin) or densely packed chromatin (heterochromatin) determines, if a given gene will be transcribed[95]. Thus, chromatin accessibility is the major determinant for gene expression, since it allows transcription factors to bind to enhancer regions and Pol II (RNA polymerase II) to transcription start site (TSS) regions, also referred to as core promoters. Chromatin accessibility, which can be determined by FAIRE-seq and ATAC-seq, is regulated on all three major levels of the epigenome, which are DNA methylation, post-translational histone modifications like methylation and acetylation and the 3-dimensional structure of chromatin[96] (**Fig. 3**). Euchromatin is found preferentially in the center of the nucleus and is composed of histone proteins that are mostly acetylated as well as of genomic DNA that has a low methylation level. In contrast, heterochromatin shows the opposite profile, *i.e.*, it is often located close to the nuclear membrane and formed by methylated histones and highly methylated DNA[97].

On the genome-wide level the collection of all epigenetic changes causes epigenomic programming of the respective tissue or cell type. Epigenomic programming events are most prominent during embryogenesis where major decision on the formation of the different tissues and organs of the embryo are taken[98]. However, epigenomic programming also occurs during differentiation of adult cells, *e.g.*, in the lifelong replacement of cells of bone marrow, colon and skin. Importantly, epigenetic changes do not cause any alterations to the genome and are mostly reversible[99].

Many epigenetic changes are the result of signal transduction cascades that are often triggered by extracellular signals, such as growth factors, cytokines and peptide hormones. In most cases, a transient signal results only in a transient epigenetic change, but the more often a signal is repeated, the more likely it causes a persistent epigenetic change. In this way, patterns of histone modifications or DNA methylation can last for days, months or even years[100]. Thus, the epigenome is able to preserve effects of cellular perturbations as epigenetic drifts[101,102]. For example, a continuous lifestyle of healthy diet and sufficient physical activity results in different epigenomes of metabolic organs than unhealthy diet combined with low physical activity. In this context, epigenomic programming is based on positive and negative learning events and represents the long-term memory of these lifestyle decisions that may even be transferred *via* epigenetic memory of germ cells to the next generation[103].

The molecular mediators of epigenetic changes are chromatin modifying enzymes that add (“write”), remove (“erase”) or interpret (“read”) post-translational histone modifications or DNA methylation[104]. These are histone acetyltransferases (HATs) and lysine

methyltransferases (KMTs) that add acetyl and methyl groups, respectively, to lysines of histone proteins. In contrast, histone deacetylases (HDACs) and lysines demethylases (KDMs) remove them. The methylation of genomic DNA at cytosines is performed by DNA methyltransferases (DNMTs), while TET (ten-eleven translocation) enzymes start the process of erasing the methyl groups by a cascade of oxidation reactions and the involvement of DNA repair enzymes. Interestingly, these chromatin modifying enzymes depend in their activity on intermediate metabolites, such as acetyl-CoA, NAD^+ (nicotinamide adenine dinucleotide) and α-ketoglutarate[105], *i.e.* the redox and metabolic state of a cell has a direct effect on its chromatin and epigenome[106,107]. Accordingly, chromatin modifying enzymes function as sensors of metabolic information, *i.e.*, if cells are in a fasting or feeding state.

In summary, chromatin accessibility is the key epigenetic determinant for the controlling gene expression. Extra- and intracellular signals, many of which derive from the exposure with nutritional molecules, are able to initiate an epigenome-wide programming process that can lead to long-term memory based on persistent chromatin changes.

Nutritional epigenomics at the example of vitamin D

The discipline nutritional epigenomics studies how dietary molecules affect gene expression *via* modulation of the epigenome[108]. Importantly, diet-induced epigenomic changes are often transient and reversible, *i.e.*, in contrast to largely irreversible cell fate decisions during cellular differentiation they are dynamic. This insight should allow to develop strategies how appropriate lifestyle decisions can lead to healthy, disease-free aging[109]. Vitamin D affects *via* its nuclear receptor VDR the epigenome of many tissues and cell types and represents a master example of nutritional epigenomics[8].

The established model of vitamin D signaling[110] suggests that VDR, like PXR, CAR, FXR, and LXR[111], is supported by RXR (retinoid X receptor) in the binding to genomic DNA. For VDR these are preferentially DR3-type response elements formed by a direct repeat of two hexameric motifs in a distance of three nucleotides (**Fig. 4**). The complete set of all genomic VDR binding sites, referred as VDR cistrome[112], had been determined by ChIP-seq in cell lines representing B lymphocytes[113], monocytes[53,114], colorectal cancer[71], hepatic stellate cells[115] and macrophages[116]. In the absence of ligand VDR binds in these cellular models to some 200-2,000 sites, while after ligand stimulation the number increases in average 2.5-fold[116]. Since the binding of transcription factors is an epigenetic effect, the ligand-dependent VDR binding to thousands of genomic sites demonstrates epigenome-wide effect of vitamin D[8].

The binding of $1,25(OH)_2D_3$ to the ligand-binding domain within VDR causes a conformational change to the receptor[117]. This has the effect that VDR loses the contact with co-repressor proteins[118], but enables the binding to co-activators proteins[119]. Some of the co-

factors have chromatin modifying activity themselves, whereas others act as a bridge to chromatin modifying enzymes. For example, in a ligand-dependent fashion VDR interacts with the chromatin modifying enzymes KDM6B and the chromatin remodeling protein BRD7 (bromodomain containing 7). Accordingly, vitamin D affects the histone markers H3K4me3 (active TSSs) and H3K27ac (active chromatin)[120,121]. In human monocytes, a stimulation with $1,25(OH)_2D_3$ affects the accessibility of some 4,500 chromatin loci within human monocytes, more than 500 of which are promoters and 2,500 are enhancer regions, as determined by FAIRE-seq and ChIP-seq[122].

VDR can bind its preferred binding motifs when they are located within accessible chromatin, *i.e.*, VDR is a "settler"-type of transcription factor. In contrast, "pioneer factors"[123] have response elements that are short enough to be accessed even in the presence of nucleosomes. The pioneer transcription factors SPI1 (also called PU.1), CEBPα and GABPα (GA binding protein transcription factor α) help VDR to access its genomic binding sites[121,124,125]. In turn, vitamin D stimulation has been shown to affect the binding of the pioneer factors to their genomic target regions[126]. Interestingly, also the *VDR* gene has been shown to be a target of epigenetic regulation[127]. This includes hypermethylation of the *VDR* gene promoter, in particular in the context of cancer, which leads to reduced VDR expression and responsiveness to vitamin D stimulation[128]. A downregulation of *VDR* expression is also observed in the context of infectious diseases, such as HIV-1 (human immunodeficiency virus 1) infection[129] and tuberculosis[130], as well as in autoimmune disorders rheumatoid arthritis[131], systemic lupus erythematosus[132] and Crohn's disease[133].

Another interesting epigenome-wide effect of vitamin D is the modulation of CTCF (CCCTC binding factor) binding at some 1,300 genomic sites[134]. The chromatin organizing protein CTCF is essential in the formation of chromatin loops, which defines the borders of the more than 2,000 topologically associated domains (TADs)[135], into which the human genome is subdivided. Importantly, a gene can be regulated by a transcription factor binding to an enhancer region, when the enhancer and the TSS of the gene are located within the same TAD. In this way, changes in chromatin looping have any effect on gene expression.

Taken together, vitamin D had been shown to affect the epigenome *via* the modulation of transcription factor binding as well as on the level of histone markers, chromatin accessibility and 3-dimensional chromatin organization.

Personalized response to vitamin D

The "big biology" project 1000 Genomes (www.internationalgenome.org) demonstrated that humans differ from each other by some 4-5 million single nucleotide variants, 0.7 million insertions/deletions and about 1,000 larger copy number variations[136]. A minor proportion of

these variants contribute to the risk for common diseases or explain the responsiveness to natural and synthetic signaling molecules. For example, effectiveness of the anti-coagulant drug warfarin is determined by variants in the genes *VKORC1* (vitamin K epoxide reductase complex subunit 1) and *CYP2C9*[137]. Thus, there are low, mid and high responders to warfarin and suggesting different doses for the prescription of the drug.

In principle, this concept seems to apply also for vitamin D[138] as suggested by the vitamin D_3 intervention studies VitDmet (NCT01479933, ClinicalTrials.gov)[139-142] and VitDbol (NCT02063334)[143,144]. Individuals were found to show a personalized reaction to vitamin D_3 supplementation, which allows them to differentiate themselves into high, mid and low responders (**Fig. 5**). On the level of vitamin D target gene regulation and other vitamin D-triggered molecular parameters, some 25% of the investigated cohorts showed to be low responders[40]. This finding implies that low responders need a higher dose of daily vitamin D_3 supplementation than suggested by population-based recommendations and guidelines. In contrast, high vitamin D responders should benefit even from a low vitamin D status and better tolerate European winters with low or no endogenous vitamin D_3 production. Therefore, high vitamin D responders should suffer less frequently from infections[145], autoimmune diseases[146] and cancer[147], because vitamin D protects against these diseases (**Fig. 5**).

The molecular basis of the vitamin D response index is not yet fully understood. In analogy to the findings about warfarin, it may be primarily explained by genetic variants. In fact, variations in the genes involved in vitamin D transport and metabolism, such as *GC*, *DHCR7* (7-dehydrocholesterol reductase), *CYP2R1* and *CYP24A1*, can explain some of the interindividual differences in the vitamin D status[148]. For example, the UV-B-driven conversion of 7-dehydrocholesterol to vitamin D_3 depends critically on *DHCR7* gene expression[149] (**Fig. 1**). Individuals with low DHCR7 activity have more 7-dehydrocholesterol in their skin and therefore a higher level of endogenously produced vitamin D_3 even at lower intensity of UV-B exposure. However, the vitamin D response index appears not depend on $25(OH)D_3$ serum levels of the investigated individuals, *i.e.*, it does not depend on respective genetic variants.

Peripheral blood mononuclear cells (PBMCs) are a mixture of B and T cells, NK (natural killer) cells and monocytes, of which the latter are the most vitamin D-responsive component. A study on the dose-dependent changes of the transcriptome of PBMCs, as determined by RNA-seq, in response to the stimulation with $1,25(OH)_2D_3$ indicated an average EC_{50}-value of 0.48 nM for 206 vitamin D target genes[150]. However, not all vitamin D target genes respond equally, but there are high responding genes like *HBEGF* (heparin binding EGF like growth factor)[151] and *G0S2*[72] that get activated already at 0.1 nM $1,25(OH)_2D_3$, while genes like *LMNA* (lamin A/C)[152] and *STAB1* (stabilin 1)[10] need concentrations of 1 nM and higher. Since ligand-dependent gene expression is an epigenetic event, the different ligand sensitivity of vitamin D

target genes suggests that interindividual differences in the vitamin D response index are also based, at least to some extent on epigenetics.

In summary, nutrigenomics of vitamin D responsiveness suggests a personalized vitamin D_3 supplementation advice. Moreover, a stratification of vitamin D intervention studies based on an individual's vitamin D response index may allow a better evaluation of the protective role of vitamin D on common diseases, such as cancer and cardiovascular disease[153].

Conclusions

Nutrition provides our body not only with molecules that serve as sources of energy[154], but some of these compounds directly communicate with our epigenome *via* the regulation of transcription factor and chromatin modifier activity[155]. The vitamin D and its metabolites are a special group of dietary molecules that have direct effects on gene regulation and therefore represents a master example of nutrigenomics. Vitamin D_3 intervention studies represent nutrigenomic experiments, in which the action of vitamin D can be investigated under human *in vivo* conditions. For example, longitudinal epigenome- and transcriptome-wide analysis, such as vitamin D-triggered changes in chromatin accessibility[156] or target gene regulation[157], can be performed with PBMCs without the need of any further *in vitro* culture.

Vitamin D connects cellular metabolism with immunity[158,159] and has in this way pleiotropic physiological impact. The daily communication between diet and the epigenomes of metabolic organs, such as in skeletal muscle, adipose tissue, pancreas and liver, modulates gene regulatory networks that keep our body in homeostasis and prevent the onset of non-communicable diseases. Therefore, personalized vitamin D_3 supplementation should be implemented in precision nutrition, in order to prevent age- and lifestyle-related diseases. This may apply in particular to disorders related to chronic inflammation.

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Figure legends

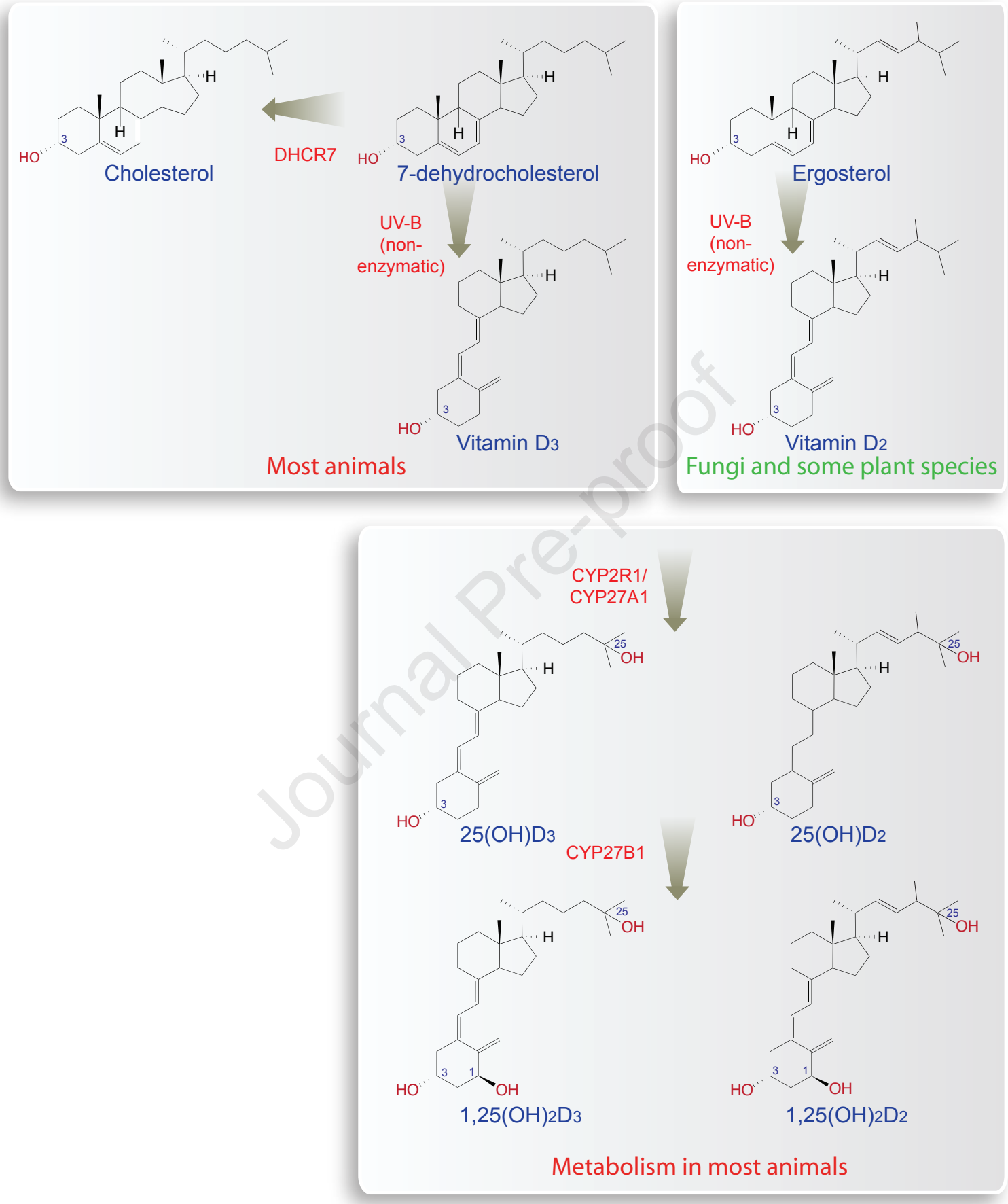
Figure 1: Synthesis of vitamin D_3 and vitamin D_2. 7-dehydrocholesterol is the second last metabolite of the cholesterol biosynthesis pathway, which also reacts into vitamin D_3, when it is exposed to UV-B (**top left**). In plants, vitamin D_2 is synthesized based on ergosterol (**top right**). The liver enzymes CYP2R1 and CYP27A1 convert both vitamin D_3 and vitamin D_2 into 25(OH)D_3 and 25(OH)D_2, respectively (**bottom**). In the kidneys, CYP27B1 add a hydroxy group to C1 of both molecules resulting in the nuclear hormones 1,25$(OH)_2D_3$ and 1,25$(OH)_2D_2$, which both active the transcription factor VDR.

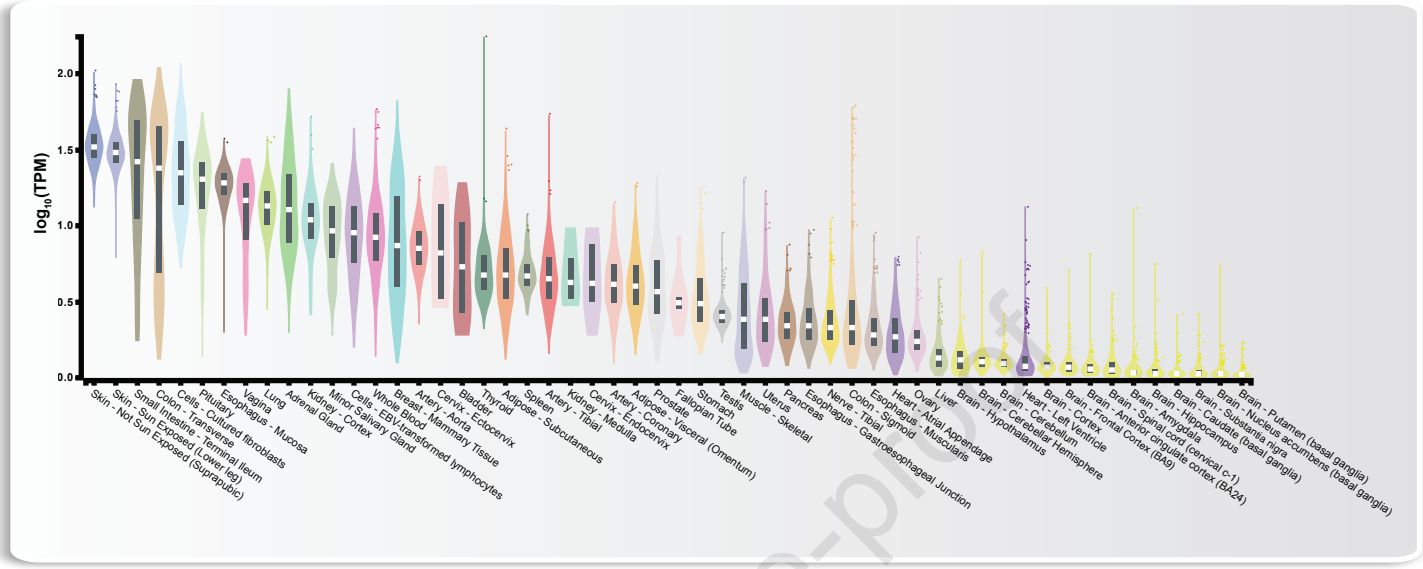
Figure 2: Expression of the *VDR* gene in 54 different human tissues. Normalized RNA-seq data are shown in TPM (transcripts per million) and sorted by descending tissue expression. Box plots display the median as well as 25th and 75th percentiles. Points indicate outliers that are 1.5 times above or below interquartile range. Data are based on GTEx analysis release V8 (dbGaP Accession phs000424.v8.p2) accessed on January 29, 2023[92].

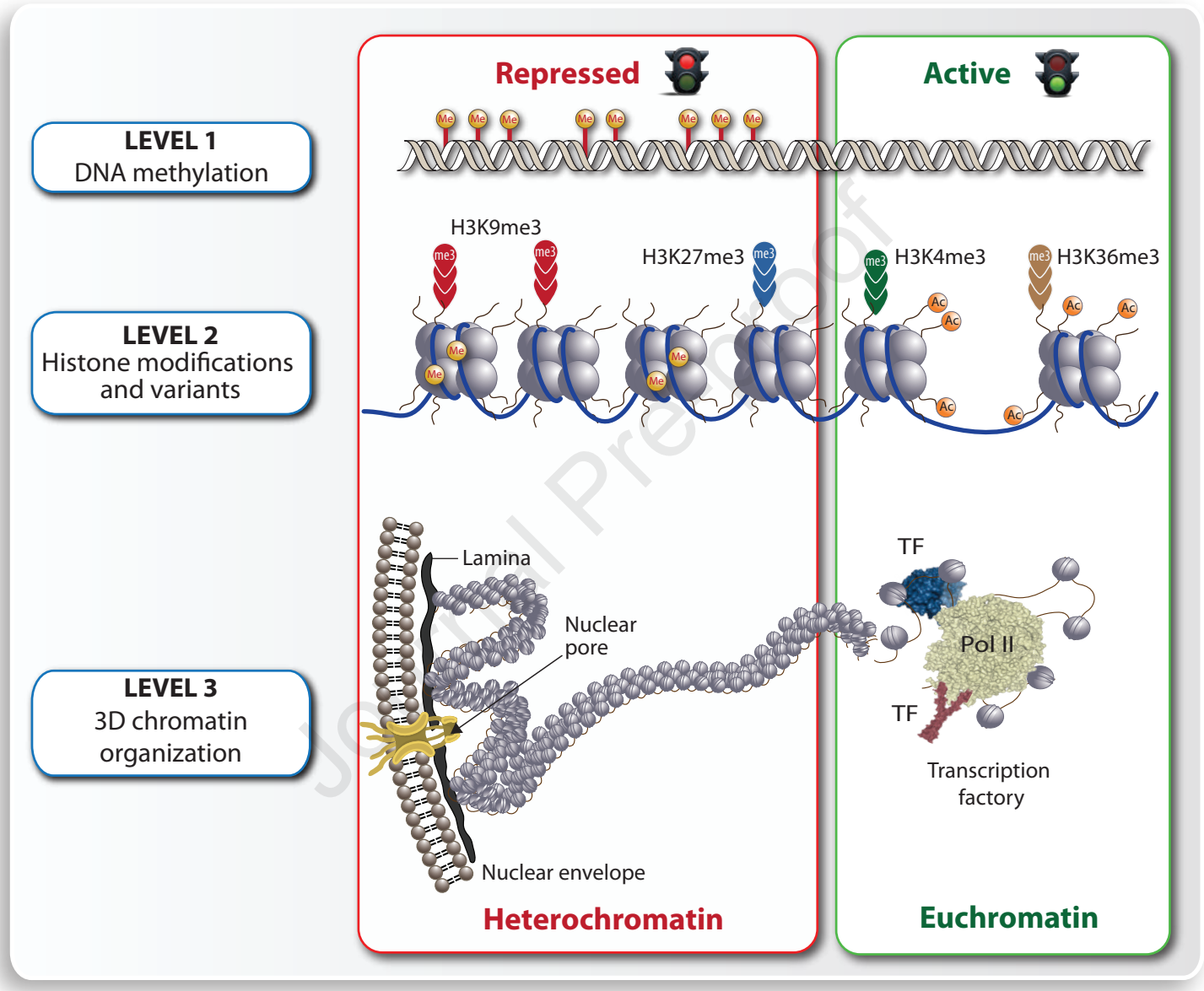
Figure 3: Epigenetic layers. Three different layers of chromatin organization are DNA methylation (**top**), histone modification (**center**) and 3-dimensional chromatin structure (**bottom**). The layers represent either heterochromatin including inactive genes (**left**) as well as euchromatin containing active genes (**right**).

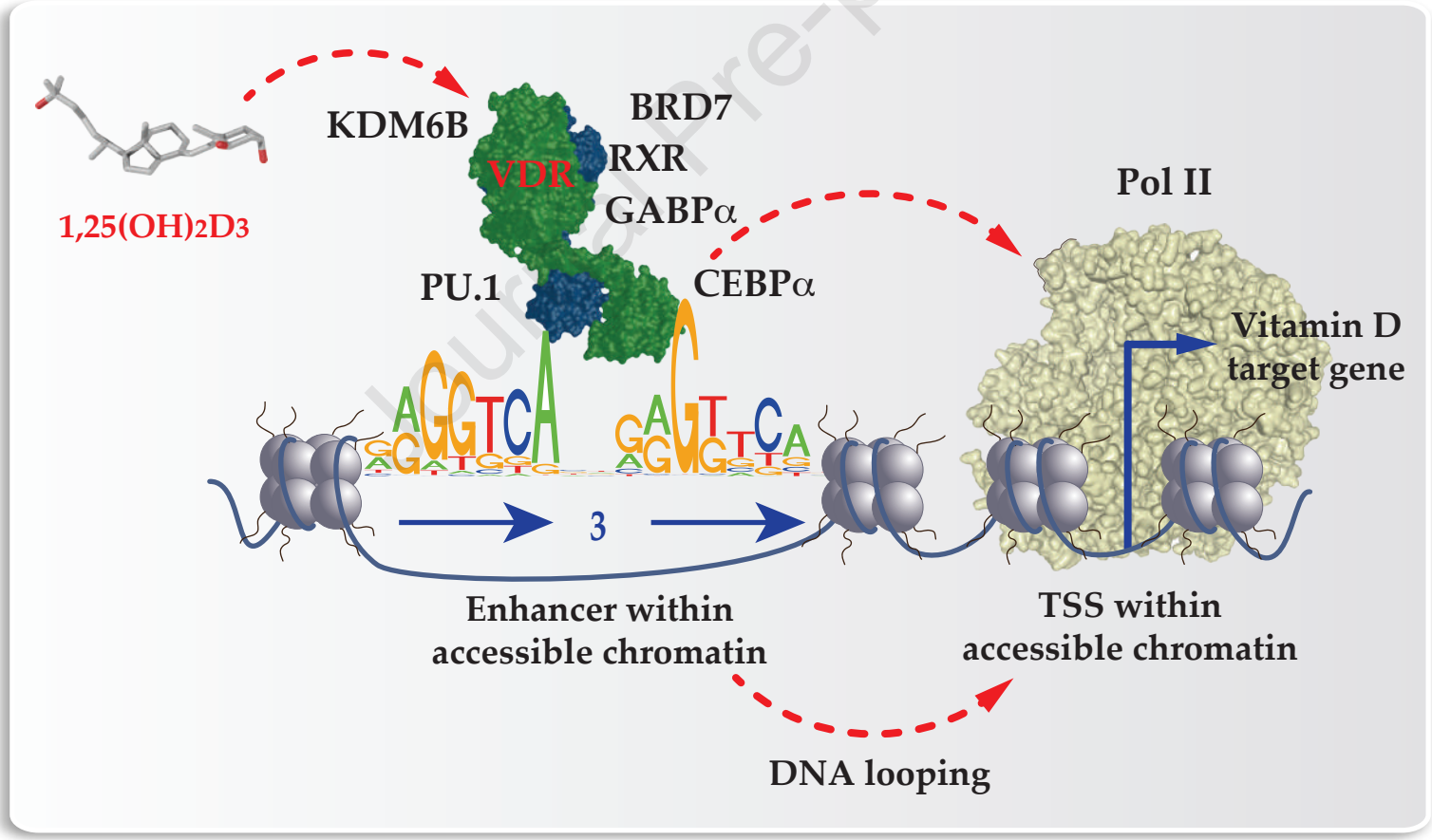
Figure 4: Principles of vitamin D signaling. VDR is activated by 1,25$(OH)_2D_3$ and interacts with a number of nuclear proteins, such as RXR, PU.1, CEBPα, GABPα, KDM6B and BRD7, and with genomic regions formed by DR3-type binding sites within enhancer regions. Activated VDR bridges *via* Mediator complex with Pol II binding to TSS regions of vitamin D target genes. In net effect, the expression of the target genes is up- or downregulated.

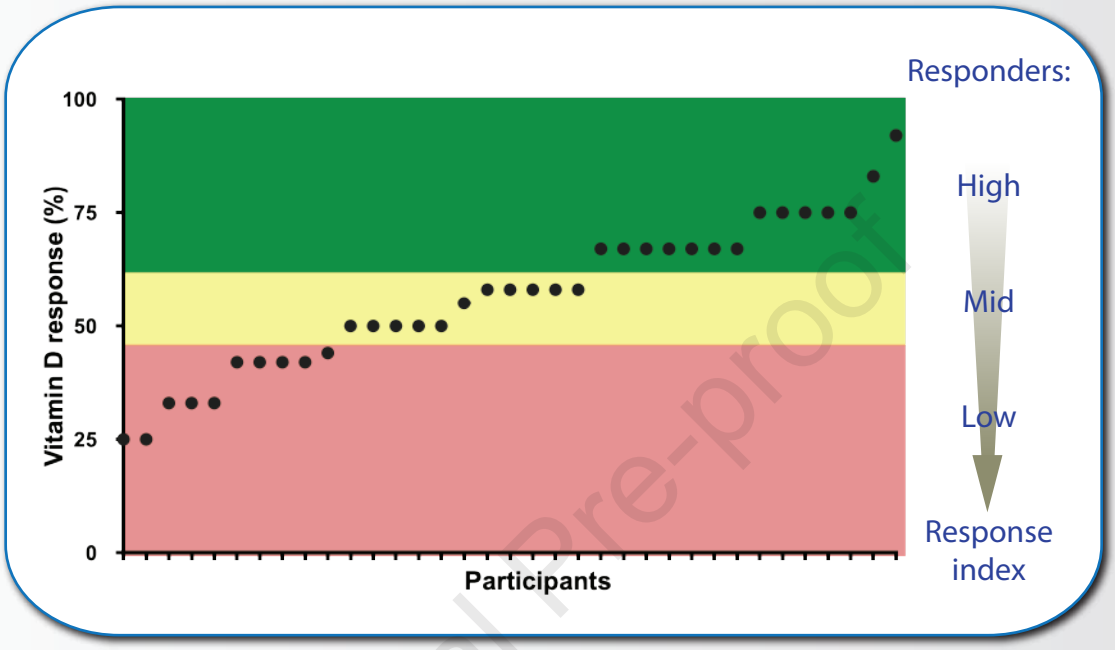
Figure 5: The vitamin D response index concept. Determining the vitamin D response index of an individual will allow personalized supplementation with vitamin D_3, in order to obtain optimal clinical benefits, such as prevention of osteoporosis, sarcopenia and autoimmune diseases.











Personalized vitamin D supplementation, in order to obtain maximal clinical benefit of vitamin D

Bone

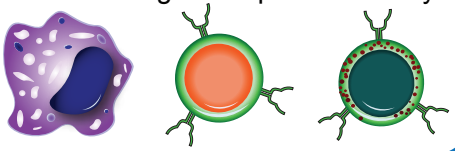
- Controlling bone homeostasis
- Calcium homeostasis

Muscle

- Strength
- Development

Immune system

- Stimmulation of innate immunity
- Controlling of adaptive immunity



Dr. Carsten Carlberg
Dr. Marianna Raczyk
Natalia Zawrotna

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The authors declare that there is no conflict of interest.