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Review

The concept of the personal vitamin D response index

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ABSTRACT

Humans are able to synthesize vitamin D_3 in their skin when exposed to UV-B, but seasonal variations, textile coverage and predominant indoor activities often make supplementation with the compound necessary. There is some dispute on the desired vitamin D status, measured via the serum concentration of the most stable vitamin D_3 metabolite, 25-hydroxyvitamin D_3, and the respective recommended daily supplementation. A possible answer may be provided by the concept of the personal vitamin D response index describing the efficiency of the molecular response to supplementation with vitamin D. The concept is based on the fact that vitamin D_3 activates via its metabolite 1α,25-dihydroxyvitamin D_3 the transcription factor vitamin D receptor and thus has a direct effect on the epigenome and transcriptome of many human tissues and cell types. Individuals can be distinguished into high, mid and low responders to vitamin D via measuring vitamin D sensitive molecular parameters, such as changes in the epigenetic status and the respective transcription of genes of mobile immune cells from blood or the level of proteins or metabolites in serum. Thus, we suggest that the need for vitamin D supplementation depends on the vitamin D status in relation to the personal vitamin D response index of an individual rather than on the vitamin D status alone.

Contents

1. Introduction

Naming vitamin D_3 a "vitamin" is misleading as every human can produce the molecule in the skin on the basis of the cholesterol precursor 7-dehydrocholesterol. However, this natural way of obtaining vitamin D_3 requires the exposure of skin to UV-B from sunlight that nowadays is insufficient for a large proportion of human population. During the last hundreds to thousand years lifestyle changes, such as preference for indoor activities and textile coverage outdoors, often resulted in insufficient sun exposure and thus low endogenous production of vitamin D_3. The most obvious consequences of the resulting vitamin D deficiency are rickets in children and osteomalacia and sarcopenia in adults [1]. In addition, numerous clinical and epidemiological studies indicated that vitamin D deficiency increases the risk for infections, autoimmune diseases, different types of cancer (breast,

Abbreviations: $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3; ChIP-seq, chromatin immunoprecipitation sequencing; COX8A, cytochrome C oxidase subunit 8A; CYP, cytochrome P450; FAIRE-seq, Formaldehyde-Assisted Isolation of Regulatory Elements sequencing; PBMC, peripheral blood mononuclear cell; PTH, parathyroid hormone; TSS, transcription start site; VDR, vitamin D receptor.

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prostate and colon), type 2 diabetes, cardiovascular diseases and other aspects of the metabolic syndrome as well as neuropsychiatric disorders [2]. Under these conditions of a dependence on external supply, vitamin D_3 is correctly termed a vitamin.

Marine plankton is producing vitamin D_3 in large amounts as a sunshield in the same UV-B-dependent, non-enzymatic reaction as in humans [3]. Therefore, fish that are at the end of the marine food chain accumulate vitamin D_3 in their liver. In addition, some fish seem to be able to use visible light from the sun, in order to produce vitamin D_3 in their skin [4]. Furthermore, some mushrooms, when exposed to UV-B, can gain reasonable amounts of vitamin D_2 [5]. In contrast, human diet that is not enriched in fatty fish or UV-exposed mushrooms is a scare source for vitamin D. Therefore, in some countries dietary products, such as milk, margarine and juices, are fortified with vitamin D_3. In addition, direct supplementation with vitamin D_3 is recommended in many countries, in particular during the winter months. However, there is some debate about the appropriate amount of daily vitamin D_3 supplementation. For example, the US Institute of Medicine [6] suggests a daily dose of 10–15 μg vitamin D_3 (400–600 IU) for children and 15–20 μg (600–800 IU) for adults, while the US Endocrine Society [7] recommends 25 μg (1000 IU) per day or more. These different recommendations refer to the dispute, which vitamin D status should be reached. The latter is determined via the serum concentration of the most stable vitamin D metabolite, 25-hydroxyvitamin D_3 ($25(OH)D_3$). The Institute of Medicine considers a level of 50 nM sufficient, while the Endocrine Society suggests at least 75 nM [6,7]. As a reference, the average $25(OH)D_3$ level of members of the traditionally living Maasai tribe in East Africa is 119 nM [8]. Since anatomically modern humans evolved some 200,000 years ago in East Africa and lived there some 150,000 years a similar lifestyle as the Maasai before some of them started to migrate north to Asia and Europe, human physiology and biochemistry should be evolutionarily well adapted to a rather high vitamin D status. It is possible that genetic adaptions during the past 10–30,000 years, such as skin lightening of Europeans, also affected average vitamin D levels in these populations. Anyhow, this argument only confirms that the human body can handle a serum $25(OH)D_3$ concentration of 120 nM, but does not prove that such a rather high vitamin D status is needed. Since tissue calcification is a possible side of overdosing vitamin D compounds [9], higher vitamin D supplementation doses are generally not recommended. However, adverse effects are generally not seen below 250 nM [10].

In this short review we are discussing that a threshold level of the vitamin D status may be insufficient in describing the need of individuals for vitamin D. Instead, we are arguing that the efficiency of the molecular response to vitamin D, referred to as the vitamin D response index, differs between individuals. We will explain that humans can be distinguished into high, mid and low responders and that their need for vitamin D supplementation depends on their vitamin D status in relation to their personal vitamin D response index.

2. The nuclear hormone vitamin D affects the human epigenome and transcriptome

In humans the molecule vitamin D_3 is biologically inert and needs to be activated by hydroxylation at positions 25 and 1 via reactions of the cytochrome P450 (CYP) enzymes CYP2R1 and CYP27B1, respectively [11]. The evolutionary perspective suggests that the resulting vitamin D_3 metabolite, 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), became a hormone when animals moved out of the water and needed to develop a stable skeleton based on calcium [12]. Therefore, the classical actions of vitamin D are the control of i) absorption of dietary calcium and phosphorus in the gut, ii) reabsorption of calcium in the renal tubules and iii) remodeling of bones. The lipophilic structure of $1,25(OH)_2D_3$ allows the molecule to pass cellular and nuclear membranes and to act in the nucleus as high-affinity ligand to the transcription factor vitamin D receptor (VDR) [13]. In this way, $1,25(OH)_2D_3$ and its precursor vitamin D_3 have a direct effect on gene regulation [14], which is a property that it shares only with a small group of other nuclear hormones, such as estrogen, testosterone and cortisol [15].

VDR is an endocrine member of the nuclear hormone receptor superfamily [16] and is involved in all molecular actions of $1,25(OH)_2D_3$, i.e. the receptor acts as the mechanistic core of vitamin D signaling. Via its DNA-binding domain VDR recognizes specific sequences within genomic DNA referred to as enhancers. Throughout the whole human genome there are at least 23,000 different VDR binding sites, the most of which are accessible in a cell-specifically fashion, as measured by chromatin immunoprecipitation coupled with massive parallel sequencing (ChIP-seq) [17]. Most of the human genome is covered by heterochromatin, i.e. in a densely packed form of chromatin, in which access of most transcription factors, such as VDR, is very much restricted [18]. This intrinsic repressive function of heterochromatin conserves the epigenetic landscape of a differentiated cell, which is composed of only 50–100,000 accessible chromatin regions per cell type [19]. Only this small subset of the human genome is accessible to VDR and can be the origin of gene regulation by vitamin D. By using the method Formaldehyde-Assisted Isolation of Regulatory Elements sequencing (FAIRE-seq), which allows the genome-wide profiling of accessible chromatin [20], we showed that nearly 9000 chromatin regions are changed in their accessibility, when THP-1 human monocytes were treated for 24 h with $1,25(OH)_2D_3$ [21]. In the same cellular model the genomic binding of the chromatin organizer CCCTC-binding factor is at more than 2100 loci sensitive to $1,25(OH)_2D_3$ stimulation [22]. Both examples demonstrate that vitamin D has a direct effect on the human epigenome.

Changes in the epigenome, such as methylation of genomic DNA or post-translational modification of nucleosome-forming histone proteins [23,24], that affect the accessibility of promoter and enhancer regions are an essential prerequisite for initiating gene transcription. The opening of VDR binding enhancers activates RNA polymerase II on the transcription start sites (TSSs) of vitamin D target genes in their vicinity and stimulates (or represses) their transcription [25]. In total, this can affect the transcription of more than 1000 genes, i.e. the stimulation of a cell with $1,25(OH)_2D_3$ results in a significant change in its transcriptome [26,27].

The *VDR* gene is expressed in most of the approximately 400 tissues and cell types of the human body suggesting that vitamin D has, via the VDR, a far wider physiological function than the control of calcium homeostasis and bone remodeling [1,6]. In fact, cells of the innate and adaptive immune system, such as monocytes, T cells and B cells [21,28,29], which are major components of peripheral blood mononuclear cells (PBMCs), are very responsive to vitamin D. Fig. 1 illustrates the example of the cytochrome C oxidase subunit 8A (*COX8A*) gene that carries a VDR binding enhancer region some 4 kb upstream of its TSS. In THP-1 cells a 24 h stimulation with $1,25(OH)_2D_3$ results in significant induction of VDR to the enhancer region and chromatin opening to the TSS region. Interestingly, also in PBMCs isolated from an individual being supplemented for one day with a bolus of vitamin D_3 (2000 μg) the chromatin at the *COX8A* TSS significantly opened. Furthermore, triplicate bolus stimulation repeats in the same individual demonstrated the significant up-regulation of *COX8A* transcription.

In summary, not only the stimulation of cell culture models with a high dose of $1,25(OH)_2D_3$ promotes genomic VDR binding and chromatin opening, but that also the supplementation of

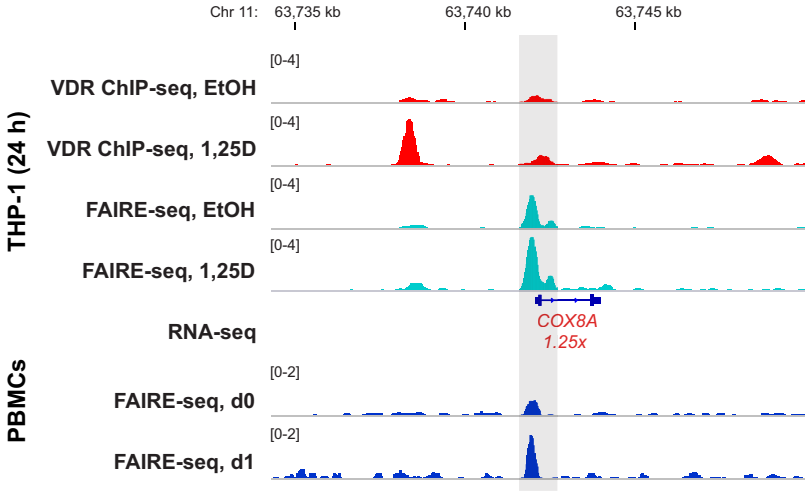

Fig. 1. Gene regulatory scenario of the *COX8A* gene. The Integrative Genomics Viewer browser [46] was used to visualize the VDR binding site 4 kb upstream of the *COX8A* TSS leading to chromatin opening at the TSS. The peak tracks display data from VDR ChIP-seq (red) and FAIRE-seq (light blue) from THP-1 cells [21] and RNA-seq and FAIRE-seq data (dark blue, unpublished results) from an individual supplemented with a vitamin D_3 bolus (2000 μg). The gene structures are shown in blue and the TSS region is shaded in grey.

individuals with the parent compound vitamin D_3 leads within the same time frame to an increase in chromatin accessibility and gene transcription.

3. Molecular interpretation of vitamin D intervention trials

The recent high public interest in vitamin D supplementation [30] and an increased understanding of the broad physiological impact of the hormone initiated during the past 10 years numerous vitamin D intervention trials. Most of these studies evaluated the health status of the participating individuals via questionnaires, medical examination or serum biochemistry, but did not perform molecular analysis on the level of changes in gene expression and chromatin accessibility. In this respect the trials VitDmet (NCT01479933) [31] and VitDbol (NCT02063334) [32,33] are exceptions and will be discussed here.

VitDmet is three-arm intervention study (daily either 0, 1600 or 3200 IU vitamin D_3) over 5 months of Finnish winter that investigated 71 elderly pre-diabetic subjects. Serum, PBMC and adipose tissue biopsies were collected at start and end of the trial and an oral glucose tolerance test was performed at both time points. In total more than 100 clinical and biochemical parameters were determined. In addition, mRNA expression of 24 vitamin D target genes was measured in PBMCs and adipocytes [34–36]. For example, one of the top ranking genes was *CD14*, which was shown previously to be most suited for describing the vitamin D status of primary blood samples [37]. In contrast to most other studies, not the differences but the ratios of the investigated parameters at end and start of the study were used for a correlation analysis with respective changes of the serum 25(OH)D_3 levels [31]. This approach demonstrated for all tested vitamin D target genes a significant correlation between changes in their mRNA expression changes and variations in the vitamin D status of the individuals [32,38]. In contrast, only 12 of the more than 100 investigated clinical and biochemical parameters, such as the well established parathyroid hormone (PTH) serum level [39], correlated well with changes in the 25(OH)D_3 levels. This provided in total 36 biomarkers for determining the vitamin D responsiveness of the individuals. Interestingly, the number of the vitamin D-triggered parameters, to which the 71 VitDmet study participants showed responsiveness, was clearly different. The least responsive individual responded only to 10 of the tested 36 parameters, while the most responsive person was positive in 30 parameters (Fig. 2**A**). By using the k-means method the 71 individuals could be segregated into 17 (23.9% of all) low responders, 36 (50.7%) mid responders and 18 (25.4%) high responders.

The VitDbol vitamin D intervention trial was designed differently from the VitDmet study, as it recruited 35 young, healthy individuals, exposed them only to one high dose of vitamin D_3 (2000 μg) and collected samples at days 0, 1, 2 and 30 [33]. As a proof-of-principle, vitamin D-triggered changes in chromatin accessibility and in serum PTH levels at three time points (d1/d0, d2/d0 and d30/d30) were used as parameters for investigating the vitamin D responsiveness of the study participants. Although with chromatin opening an epigenomic variation, and not a transcriptomic change, was determined and in total only 12 values per individual were measured, sufficient data were acquired, in order to distinguish the 35 VitDbol participants into 10 (28.6%) low responders, 11 (31.4%) mid responders and 14 (40.0%) high responders (Fig. 2**B**).

Taken together, both a long-term daily vitamin D intervention study (VitDmet) as well as a short-term vitamin D bolus trial (VitDbol) allows segregating the study participants into high, mid and low responders. A wide range of vitamin D-triggered parameters, such as changes of gene expression, chromatin accessibility and serum proteins and metabolites, showed to be suitable for determining the vitamin D response index.

4. The vitamin D response index

The results of intervention trials VitDmet and VitDbol demonstrate that humans differ in their molecular response to vitamin D supplementation. Every individual has a personal vitamin D response index: high responders have a high index, while low responders have a lower index. Thus, the vitamin D response index is an (epi)genetic property of an individual that may not change at all or only slowly during the development of an age-related disease. VitDmet and VitDbol provide a non-exclusive list of examples, how the vitamin D response index could be determined. The only essential conditions is to obtain blood samples from the same individual at two or more time points of a period, in which the vitamin D status of the person changes. This could be the fast raise of 25(OH)D_3 serum concentrations after a vitamin D bolus or more long-term improvements of the vitamin D status based on

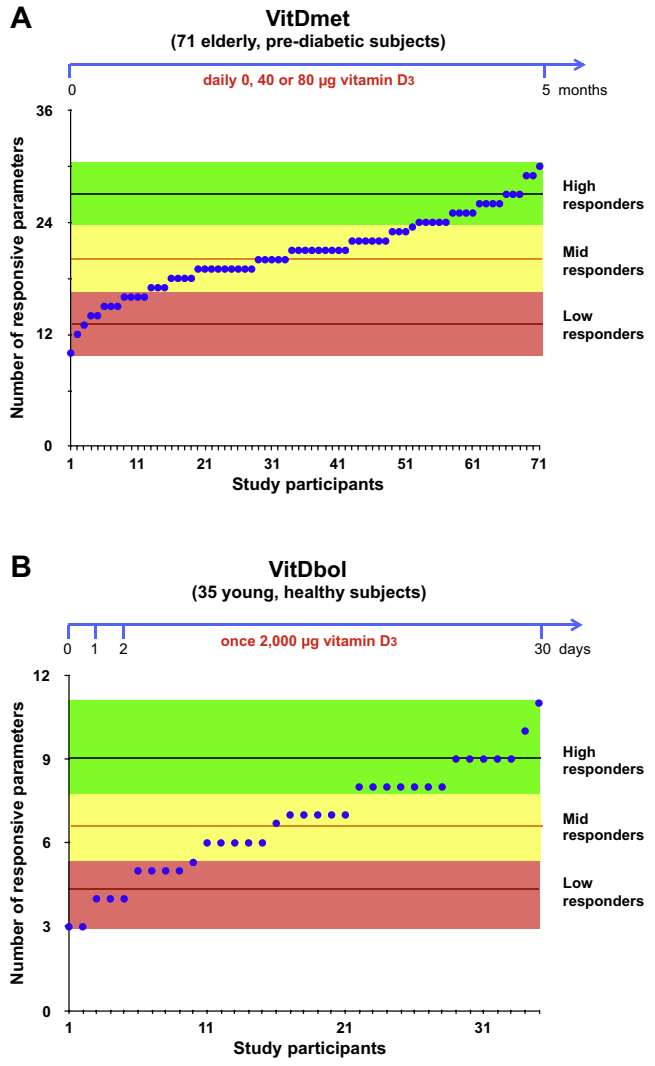

Fig. 2. Vitamin D responsiveness. VitDmet (**A**) and VitDbol (**B**) are vitamin D intervention trials with a rather different design. In VitDmet 71 elderly (>60 years), pre-diabetic subjects were supplemented daily over 5 months with three different vitamin D_3 doses [31], while in VitDbol 35 young (18–30 years) healthy subjects were treated once with a bolus of 2000 µg vitamin D_3 [32]. PBMC and serum samples were collected at months 0 and 5 (VitDmet) or at days 0, 1, 2 and 30 (VitDbol). The vitamin D responsiveness of the study participants was determined on the basis of vitamin D-triggered changes in the expression of 24 target genes in PBMCs and 12 clinical and biochemical parameters (36 in total, **A**) or changes of chromatin accessibility at 3 genomic regions and PTH levels at each 3 different time points (12 in total, **B**). The number of responsive parameters (blue data points) is indicated for each study participant. A k-means clustering approach was used to distinguish high responders (green), from mid responders (yellow) and low responders (red). Data were collected from original reports [32,33].

daily supplementations. In fact, most critical are changes in the vitamin D status that are correlated with any of the established molecular parameters of vitamin D responsiveness. Thus, also natural increases of the vitamin D status due to UV-B exposure during summer or declines of insufficiently supplemented persons during winter are suited for vitamin D response index determinations.

The genetic origin of all contemporary humans is East Africa, i.e. an equatorial region with no seasonal changes in UV-B exposure. This implies that human physiology is more likely adapted to a constant vitamin D status than to 25(OH)D_3 serum level changes between summer and winter. However, also in naturally living populations, such as the Maasai, the vitamin D status shows a wide personal range with 25(OH)D_3 serum concentrations between 58 and 167 nM [8]. The agricultural revolution some 5–10,000 years ago and in particular the industrial revolution of the last 100–200 years drastically changed the lifestyle of nearly all human populations concerning biochemical and physiological parameters, such as physical activity, the composition of diet and the intestinal microbiome. These changes resulted in homeostatic imbalances that are the basis of many common non-communicable disorders, such as diabetes, autoimmune disease and cancer. The increase of these non-communicable diseases inversely correlates with the in average low rate of endogenous vitamin D_3 production of humans that are genetically still mainly adapted to the environmental conditions and lifestyle of their ancestors in the savannahs of East Africa.

Individuals that are very responsive to vitamin D, i.e. have a high vitamin D response index, may benefit even from low vitamin D serum concentrations, i.e. they are assumed to support well a rather low vitamin D status. Therefore, individuals with a high vitamin D response index should less likely be affected by those non-communicable disorders against which vitamin D has a protective function. In contrast, persons with a low vitamin D response index should aim on a high vitamin D status, in order to still obtain maximal benefit from the disease protective role of vitamin D. This suggests that the dose of daily vitamin D supplementation should be adapted to the vitamin D response index of the individual.

Many vitamin D intervention trials were inconclusive concerning a disease-preventive function of the nuclear hormone, so that the Institute of Medicine was unable to provide recommendations for non-musculoskeletal effects of vitamin D [6]. Significant effects of vitamin D_3 supplementation to low responders may be diluted by the lack of disease-related response of mid and high responder that do not need any additional vitamin D. In addition, the vitamin D status of participants of most observational studies is based on one single measurement of the 25(OH)D_3 serum level. Thus, in future a segregation of participants of vitamin D intervention trials into low, mid and high responders may result in more conclusive results and may resolve some of the debate on recommended vitamin D supplementation for different groups of human populations.

In summary, results of the VitDmet and VitDbol trials suggest some 25% that of the human population, i.e. close to 2 billion humans, are low vitamin D responders and accordingly display a low vitamin D response index. These predictions are independent of the geographic location of an individual, i.e. whether he/she may live in a sunny country or not, and suggest that such a person is vulnerable by insufficient vitamin D supplementation.

5. How to use the vitamin D response index?

As an example of a high rate of vitamin D deficiency, we reported recently the vitamin D status of 60,979 patients (57.5% females, 42.5% males) admitted to the Burjeel Hospital in Abu Dhabi, United Arab Emirates (UAE) from October 2012 to September 2014 [40]. From these patients 82.5% showed insufficiency (25(OH)D_3 serum levels <75 nM) or even vitamin D deficiency (25(OH)D_3 serum levels <50 nM) and 26.4% of the females and 18.4% of the males even had extreme vitamin D deficiency (25(OH)D_3 serum levels <25 nM). Moreover, teenagers (13–19 years) showed the lowest vitamin D status. This confirms other studies reporting that, despite abundant sunshine, the prevalence of hypovitaminosis D is high, especially among women, in the populations of UAE, Saudi Arabia and other Middle Eastern countries [41]. In general, 86.1% UAE nationals and 78.9% visitors of other nationalities have an insufficient vitamin D status including

28.4% and 17.5% of persons displaying extreme vitamin D deficiency. This may contribute to the non-communicable diseases hypertension, diabetes and obesity that are widespread in the Middle Eastern region [42,43]. Although there seems to be an easy solution to the vitamin D deficiency problem in this region of the world, i.e. more exposure of bare skin to the abundant sunshine, cultural traditions and extreme heat during the summer months prevent this option. Therefore, supplementation with appropriate doses of vitamin D_3 is recommended.

Our vitamin D intervention studies VitDmet and VitDbol both indicated that some 25% of the study participants are low responders. When we extrapolate this observation to other populations, also some 25% of persons living in the Middle Eastern region may be low responders. A screening using a single vitamin D bolus treatment and measurements at days 0 and 2 paired with a simplified protocol of the molecular analysis used in the VitDbol study [33], may be the easiest way to identify persons with a low vitamin D response index. At least these persons should then be supplemented to a sufficient vitamin D status (probably 25(OH)D_3 serum levels in the order of 75–100 nM) [44].

When a person's vitamin D response index is known, a personalized vitamin D supplementation protocol can be designed directing to an optimal vitamin D status. For example, a smartphone app may integrate the vitamin D response index with the vitamin D intake by diet, such fatty fish or fortified food, outdoor physical activity correlating with sun exposure and adiposity that decreases 25(OH)D_3 bioavailability. This will rather accurately recommend a person's vitamin D intake and will show daily adaptions. Studies with individuals having a stable optimized vitamin D status will have a higher probability to prove or disprove claims about the disease preventive impact of vitamin D than previous studies.

The molecular basis of individual's differences in their vitamin D response indices is presently unsolved. In case of the vitamin D status some single nucleotide polymorphisms (SNPs) of individuals were found to correlate with a higher or a lower serum 25(OH)D_3 level [45]. Accordingly, variations in the vitamin D response index may be in part based on genetic variations. Respective SNPs need to be identified by genome-wide association studies or whole genome sequencing of a larger group of subjects (>1000). However, in analogy to most traits underlying common diseases, such as type 2 diabetes or cardiovascular disease, genetic variations, e.g. in vitamin D metabolizing enzymes or transporters, may predict only approximately 20% of the risk for a low vitamin D response index, while the remaining risk may be rather explained on the basis of variations in the epigenome. The epigenome responds to environmental changes and depends on the individual's lifestyle. This implies that the vitamin D response index may change due to aging or the onset of a disease, such as the metabolic syndrome.

6. Conclusions

The molecular analysis of the vitamin D supplementation trials VitDmet and VitDbol suggests that humans differ in their response to vitamin D, i.e. a personal vitamin D response index can be assigned to each individual. This dynamic response to vitamin D often does not correlate with the static description of the vitamin D status. This implies that the latter alone may represent an insufficient description of the impact of vitamin D for an individual. Therefore, everyone should not only be aware of his/her vitamin D status but also about his/her vitamin D response index. Finally, we recommend using the vitamin D response index for the stratification of study cohorts, in order to challenge observational studies suggesting that high serum concentrations of vitamin D protect against cardiovascular disease, diabetes, colorectal cancer and all-cause mortality.

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