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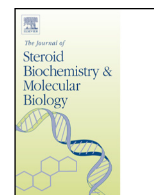

Dissecting high from low responders in a vitamin D_3 intervention study

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ABSTRACT

Vitamin D_3 is a pleiotropic signaling molecule that has via activation of the transcription factor vitamin D receptor (VDR) a direct effect on the expression of more than 100 genes. The aim of this study was to find transcriptomic and clinical biomarkers that are most suited to identify vitamin D_3 responders within 71 pre-diabetic subjects during a 5-month intervention study (VitDmet). In hematopoietic cells, the genes *ASAP2*, *CAMP*, *CD14*, *CD97*, *DUSP10*, *G0S2*, *IL8*, *LRRC8A*, *NINJ1*, *NRIP1*, *SLC37A2* and *THBD* are known as primary vitamin D targets. We demonstrate that each of these 12 genes carries a conserved VDR binding site within its genomic region and is expressed in human peripheral blood mononuclear cells (PBMCs). The changes in the expression of these genes in human PBMCs at the start and the end of the vitamin D-intervention were systematically correlated with the alteration in the circulating form of vitamin D_3, 25-hydroxyvitamin D_3 (25(OH)D_3). Only 39–44 (55–62%) of the study subjects showed a highly significant response to vitamin D_3, i.e., we considered them as "responders". In comparison, we found for 37–53 (52–75%) of the participants that only 12 biochemical and clinical parameters, such as concentrations of parathyroid hormone (PTH) and insulin, or computed values, such as homeostatic model assessment and insulin sensitivity index, show a correlation with serum 25(OH)D_3 levels that is as high as that of the selected VDR target genes. All 24 parameters together described the pleiotropic vitamin D response of the VitDmet study subjects. Interestingly, they demonstrated a number of additional correlations that define a network, in which PTH plays the central role. In conclusion, vitamin D_3-induced changes in human PBMCs can be described by transcriptomic and serum biomarkers and allow a segregation into high and low responders.

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1. Introduction

The endocrine system of vitamin D involves (i) vitamin D_3 that is produced in the skin from 7-dehydrocholesterol with energy provided by the UV-B component of sun light [1], (ii) 25-hydroxyvitamin D_3 (25(OH)D_3), which is the most abundant vitamin D metabolite and the widely accepted indicator of the vitamin D status within the human body [2], and (iii) 1,25-dihydroxyvitamin D_3 (1,25(OH)$_2D_3$), which is a specific high-affinity ligand of the transcription factor VDR [3]. The classical physiological function of vitamin D is the regulation of calcium and phosphate homeostasis and its impact on bone mineralization [4].

Abbreviations: 1,25(OH)$_2D_3$, 1α,25-dihydroxyvitamin D_3; 25(OH)D_3, 25-dihydroxyvitamin D_3; ADIPOQ, adiponectin; ASAP2, ArfGAP with SH3 domain, ankyrin repeat and PH domain 2; B2M, beta-2-microglobulin; CAMP, cathelicidin antimicrobial peptide; CD14, CD14 molecule; CD97, CD97 molecule; ChIP, chromatin immunoprecipitation; ChIP-seq, ChIP sequencing; DR3, direct repeat spaced by 3 nucleotides; DUSP10, dual specificity phosphatase 10; FFA, free fatty acid; G0S2, G0/G1switch 2; GAPDH, glycerinaldehyde-3-phosphate-dehydrogenase; GEO, gene expression omnibus; GTP, glutamic-pyruvate transaminase (alanine aminotransferase); HOMA, homeostatic model assessment; HPRTI, hypoxanthine phosphoribosyltransferase 1; IGV, integrative genomics viewer; IL6, interleukin 6; IL8, interleukin 8; INS, insulin; IR, insulin resistance; LPS, lipopolysaccharide; LRRC8A, leucine rich repeat containing 8 family, member A; NINJ1, ninjurin 1; NRIP1, nuclear receptor interacting protein 1; PBMC, peripheral blood mononuclear cell; PTH, parathyroid hormone; qPCR, real-time quantitative polymerase chain reaction; RPLP0, ribosomal protein, large, P0; SLC37A2, solute carrier family 37, member 2; TNFRSF1B, tumor necrosis factor receptor superfamily, member 1B; TSS, transcription start site; THBD, thrombomodulin; VDR, vitamin D receptor.

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However, vitamin D_3 is a pleiotropic signaling molecule, which via $1{,}25(OH)_2D_3$ and VDR regulates numerous physiological processes ranging from the control of cellular growth, intracellular metabolism, and innate and adaptive immunity [5,6].

In order to achieve an optimal vitamin D status, sufficient exposure to natural UV-B radiation or, instead, adequate intake from diet or supplements is needed. However, serum $25(OH)D_3$ levels show high inter-individual variations that are based on age, sun exposure, diet, level of obesity and (epi) genetic polymorphisms [7–9]. The US Institute of Medicine recommends a serum $25(OH)D_3$ concentration of 50 nM [10], but other scientists suggest 75 nM or even more [11]. Based on these threshold definitions worldwide billions of people have to be considered as vitamin D deficient. As a consequence, in these deficient individuals not only the bone health is compromised but vitamin D insufficiency is also associated with a number of diseases, such as cancer, autoimmune disorders and all components of the metabolic syndrome [12]. In those cases where a sufficient vitamin D status cannot be achieved by appropriate sun exposure or dietary changes, a direct supplementation with vitamin D_3 seems to be the most straightforward intervention. However, the inter-individual variation suggests that a "one-size-fits-all" approach is not suited for vitamin D_3 supplementation [13].

VDR belongs to the nuclear receptor transcription factor superfamily, whose members are directly activated by small lipophilic compounds [14]. Insight into the genome- and transcriptome-wide actions of VDR and $1{,}25(OH)_2D_3$ can help in a more accurate evaluation of the human individual's responsiveness to, and needs for, vitamin D_3. Transcriptome-wide analysis indicated that depending on cell type between 200 and 600 genes are primary targets of $1{,}25(OH)_2D_3$ [15–18]. The chromatin immunoprecipitation (ChIP) method coupled with massive parallel sequencing (ChIP-seq) allows the genome-wide mapping of nuclear proteins to their genomic locations [19]. VDR ChIP-seq data had been published for the human cellular models (i) GM10855 and GM10861 lymphoblastoid cells [15], (ii) THP-1 monocyte-like cells [16], (iii) lipopolysaccharide (LPS)-differentiated macrophage-like THP-1 cells [20], (iv) LS180 colorectal cancer cells [21] and (v) LX2 hepatic stellate cells [22]. A re-analysis of these data using identical peak calling settings identified genome-wide more than 23,000 individual VDR loci, of which more than 70% are specific for one cellular system [20]. This indicates that vitamin D signaling is largely cell-type specific.

In order to determine how the vitamin D responsiveness of human individuals could be determined most accurately, in this study we investigated changes in the expression of primary VDR target genes after vitamin D_3 supplementation. Since PBMCs are the easiest available vitamin D responsive human cell types to be achieved, we took advantage of already existing samples from 71 participants of the vitamin D_3 intervention trial VitDmet. We selected the primary VDR target genes ArfGAP with SH3 domain, ankyrin repeat and PH domain 2 (*ASAP2*), cathelicidin antimicrobial peptide (*CAMP*), CD14 molecule (*CD14*), CD97 molecule (*CD97*), dual specificity phosphatase 10 (*DUSP10*), G0/G1switch 2 (*G0S2*), interleukin 8 (*IL8*), leucine rich repeat containing 8 family, member A (*LRRC8A*), ninjurin 1 (*NINJ1*), nuclear receptor interacting protein 1 (*NRIP1*), solute carrier family 37, member 2 (*SLC37A2*) and thrombomodulin (*THBD*) and investigated their expression on PBMCs obtained before and after the VitDmet trial in correlation to changes in serum $25(OH)D_3$ concentrations. In parallel, we tested some 200 biochemical and clinical parameters from the same study participants for a possible correlation with alterations in $25(OH)D_3$ levels, out of whom we found exactly 12 to be significantly correlated. All 24 parameters together described the pleiotropic actions of vitamin D in the VitDmet study subjects and allow their segregation into high and low responders.

2. Material and methods

2.1. ChIP-seq data visualization

The integrative genomics viewer (IGV) [23] was used to visualize the VDR ChIP-seq data, which had been summarized by Tuoresmäki et al. [20], which is accessible at Gene Expression Omnibus (GEO, www.ncbi.nlm.nih.gov/geo) under the accession number GSE53041.

2.2. Samples of the VitDmet study

The inclusion criteria of the 73 participants of the VitDmet study (NCT01479933, ClinicalTrials.gov) were (i) to be ≥60 years of age, (ii) to show evidence of disturbed glucose homeostasis, such as impaired fasting glucose or impaired glucose tolerance, but not yet manifest type 2 diabetes, and (iii) to have a body mass index between 25 and 35. Out of these 73 subjects, we selected those 71, for whom PBMC isolates both from the start and the end of the trial were available. The research ethics committee of the Northern Savo Hospital District had approved the study protocol (#37/2011). All participants gave a written informed consent to participate in the study (for further details see [24]).

Serum concentrations for $25(OH)D_3$ were measured from venous blood samples using a high performance liquid chromatography with coulometric electrode array as described previously [25]. Moreover, serum protein levels for the bone health marker parathyroid hormone (PTH), the soluble tumor necrosis factor receptor superfamily, member 1B (TNFRSF1B), the cytokine interleukin 6 (IL6), the adipocyte cytokine adiponectin (ADIPOQ) and the liver enzyme alanine aminotransferase (GTP) were determined by standard methods as described previously [25]. Other biochemical parameters were assayed at a local laboratory service provider (ISLAB, Kuopio, Finland).

At the start and the end of the 5-month intervention a 2 h oral glucose tolerance test was carried out with 75 g glucose; at time points 0, 30 and 120 min glucose, free fatty acids (FFA) and insulin (INS) were measured. Homeostatic modeling assessment (HOMA) indices were computed according to the nonlinear function using the approach of Wallace et al. [26], while the insulin sensitivity index was calculated as described by Matsuda and DeFronzo [27].

2.3. qPCR from PBMC samples

PBMCs were isolated, RNA was extracted and cDNA synthesized as described previously [24,28]. qPCR reactions were performed using 280 nM of reverse and forward primers (Table S1), 1/20 diluted cDNA template and LightCycler 480 SYBRGreen I Master mix (Roche). In the PCR reaction the hotstart Taq polymerase was activated for 10 min at 95 °C, followed by 43 amplification cycles of 20 s denaturation at 95 °C, 15 s annealing at primer-specific temperatures (Table S1) and 15 s elongation at 72 °C and a final elongation for 10 min at 72 °C. PCR product specificity was monitored using post-PCR melt curve analysis. Relative mRNA expression levels were determined using the formula $2^{-(\Delta Ct)}$, where ΔCt is $Ct_{(targetgene)}$ − mean of $Ct_{(referencegenes)}$. The four internal reference genes beta-2-microglobulin (*B2M*), glycerinaldehyde-3-phosphate-dehydrogenase (*GAPDH*), hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) and ribosomal protein, large, P0 (*PRLP0*) were used as references. The stability of the expression of the reference genes was determined using the geNorm algorithm [29].

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