Primary Vitamin D Target Genes Allow a Categorization of Possible Benefits of Vitamin D₃ Supplementation

Carsten Carlberg¹*, Sabine Seuter¹, Vanessa D. F. de Mello², Ursula Schwab²,³, Sari Voutilainen², Kari Pullki⁴, Tarja Nurmi⁵, Jyrki Virtanen⁵, Tomi-Pekka Tuomainen⁵, Matti Uusitupa²,⁶

¹ School of Medicine, Institute of Biomedicine, University of Eastern Finland, Kuopio, Finland, ² Institute of Public Health and Clinical Nutrition, Department of Clinical Nutrition, University of Eastern Finland, Kuopio, Finland, ³ Institute of Clinical Medicine, Internal Medicine Kuopio University Hospital, Kuopio, Finland, ⁴ Eastern Finland Laboratory Centre and Department of Clinical Chemistry, University of Eastern Finland, Kuopio, Finland, ⁵ Institute of Public Health and Clinical Nutrition, Department of Public Health, University of Eastern Finland, Kuopio, Finland, ⁶ Research Unit, Kuopio University Hospital, Kuopio, Finland

Abstract

Vitamin D deficiency has been associated with an increased risk of developing a number of diseases. Here we investigated samples from 71 pre-diabetic individuals of the VitDmet study, a 5-month high dose vitamin D₃ intervention trial during Finnish winter, for their changes in serum 25-hydroxyvitamin D₃ (25(OH)D₃) concentrations and the expression of primary vitamin D target genes in peripheral blood mononuclear cells and adipose tissue. A negative correlation between serum concentrations of parathyroid hormone and 25(OH)D₃ suggested an overall normal physiological vitamin D response among the participants. The genes CD14 and thrombomodulin (THBD) are up-regulated primary vitamin D targets and showed to be suitable gene expression markers for vitamin D signaling in both primary tissues. However, in a ranking of the samples concerning their expected response to vitamin D only the top half showed a positive correlation between the changes of CD14 or THBD mRNA and serum 25(OH)D₃ concentrations. Interestingly, this categorization allows unmasking a negative correlation between changes in serum concentrations of 25(OH)D₃ and the inflammation marker interleukin 6. We propose the genes CD14 and THBD as transcriptomic biomarkers, from which the effects of a vitamin D₃ supplementation can be evaluated. These biomarkers allow the classification of subjects into those, who might benefit from a vitamin D₃ supplementation, and others who do not.

Introduction

Vitamin D is a fat-soluble vitamin that can be obtained by diet but is mainly synthesized from 7-dehydrocholesterol in an UV-B-dependent reaction in the skin [1]. In the liver, vitamin D₃ is then further converted into 25-hydroxyvitamin D₃ (25(OH)D₃), which is the major storage form of vitamin D. In the kidney, 25(OH)D₃ is further 1α-hydroxylated to yield its hormonal form, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), which acts as a ligand to the transcription factor vitamin D receptor (VDR) [2–4]. Serum 25(OH)D₃ concentration is the widely accepted indicator of the vitamin D status for the human body [5]. Vitamin D deficiency is defined as a serum 25(OH)D₃ concentration of below 50 nM (20 ng/ml) [6] and affects more than 1 billion humans, i.e. it is one of most common health risks [7]. Vitamin D deficiency is caused by the lack of adequate vitamin D in diet and an insufficient exposure to sun [8]. The musculoskeletal consequences of inadequate vitamin D concentrations are well established and include rickets in children and osteomalacia and fractures in adults [9]. A growing number of other diseases, such as type 1 and type 2 diabetes, cardiovascular disease and cancers of the breast, prostate and colon, have also been linked to vitamin D insufficiency, but causal associations have not yet been fully established [10–14]. In contrast, high vitamin D concentrations can cause overcalcification of bones, soft tissues, heart and kidneys leading to kidney stones and hypertension [15], but these side effects occur only at 25(OH)D₃ concentrations above 240 nM [16].

The classical, physiological role of 1,25(OH)₂D₃ and its receptor is the regulation of calcium and phosphate homeostasis and bone mineralization [17], but there is also a lot of evidence that VDR ligands have anti-proliferative and immuno-modulatory functions [18,19]. This fits both with the widespread expression of the VDR and the above described consequences of vitamin D deficiency. Transcriptome-wide analysis indicated that per cell type between 200 and 600 genes are primary targets of vitamin D [20–23]. Since most of these genes respond to vitamin D in a cell-specific fashion, the total number of vitamin D targets in the human genome is far higher than 1,000. This is supported by the genome-wide view on VDR binding sites in human lymphoblastoids [20], THP-1 human monocytic leukemia cells [21] and LS180 human colorectal cancer cells [24] obtained by chromatin immunoprecipitation combined with high throughput sequencing (ChIP-seq). These studies reported between 1,600 and 2,800 genomic VDR binding sites that locate both up- and downstream of the transcription start sites of primary vitamin D target genes. However, the three ChIP-seq
datasets overlap only at some 200 genomic locations [25], i.e. there is a limited set of conserved targets of vitamin D and its receptor also on the genomic level. Sufficient exposure to natural UV-B radiation or adequate intake from diet or supplements is needed to achieve an optimal serum 25(OH)D3 concentration. However, the change in serum 25(OH)D3 concentrations can vary widely from person to person. Diet and sun exposure together with age and adiposity in average account only for some 30% of the inter-individual variation in average 25(OH)D3 serum concentrations [26]. Accordingly, genetic and epigenetic factors are responsible for the main variation in vitamin D concentrations [27–29]. Based on this wide inter-individual response variation, it is obvious that a “one-size-fits-all” approach will not work ideally for vitamin D supplementation. Therefore, we investigated in this study samples from 71 pre-diabetic participants of the VitDmet cohort, a 5-month high dose vitamin D3 intervention trial during Finnish winter [30], for their changes in serum 25(OH)D3 concentrations and the expression of primary vitamin D target genes in peripheral blood mononuclear cells and adipose tissue. Only the top half of a ranking concerning response to vitamin D provided a significant correlation between the changes of CD14 or THBD mRNA and serum 25(OH)D3 concentrations. We present CD14 and THBD as transcriptomic biomarkers, from which straightforward conclusions on the benefits of a vitamin D3 supplementation can be obtained.

Materials and Methods

VitDmet study design and ethics statement
Participants donating PBMCs and adipose tissue samples had been participating in the VitDmet study (NCT01479933, ClinicalTrials.gov). They were selected to be ≥60 years of age for males or ≥65 for females, showed evidence of disturbed glucose homeostasis, i.e. impaired fasting glucose or impaired glucose tolerance, but no type 2 diabetes, and had a body mass index (BMI) between 25 and 35. An additional inclusion criterion was a requirement for participant baseline serum 25(OH)D3 concentration of below 75 nM [30]. The research ethics committee of the Northern Savo Hospital District had approved the study protocol. All participants gave a written informed consent to participate in the study.

Serum measurements
Serum 25(OH)D3 concentrations were measured from venous blood samples using a high performance liquid chromatography with coulometric electrode array as described previously [31]. Only a few study participants had a quantifiable serum concentration of 25(OH)D3, which is of plant origin; therefore only 25(OH)D3 was considered. Serum concentrations of PTH were assayed by a chemiluminescence kit (DiaSorin, Diethzentbach, Germany) and those of IL6 by ELISA (Quantikine Kit; R&D Systems, Minneapolis, MN, USA). Serum calcium was determined with colorimetric Arsenazo III test using a Konelab 20XT clinical chemistry analyzer (Thermo Fisher Scientific).

PBMC isolation, adipose tissue biopsy and sample preparation
Blood samples for PBMC isolation and adipose tissue samples were collected after overnight (12 h) fasting of the study participants. PBMCs were isolated from 8 ml of peripheral blood in a Vacutainer CPT Cell Preparation Tube with sodium citrate (BD, Franklin Lakes, NJ, USA) according to the manufacturer’s instructions and stored at −80°C until used for RNA extraction [32]. Adipose tissue (0.5–1 g) was taken by needle biopsy from subcutaneous abdominal adipose tissue under local anesthesia (10 mg/ml lidocaíne without adrenalin). The adipose tissue samples were washed twice with phosphate-buffered saline, frozen quickly in liquid nitrogen and stored at −80°C until used for RNA extraction.

RNA extraction and cDNA synthesis
Total RNA from PBMCs and adipose tissue was extracted using the TRIzol method followed by further purification with miRNeasy Mini Kit columns according to the instructions provided by the manufacturer (Qiagen). The RNA concentration and the A260/A280 ratio were measured using the NanoDrop spectrophotometer, an acceptable ratio being 1.9–2.1 [33]. The total RNA was reverse transcribed into cDNA using the High-Capacity cDNA Archive Kit (Applied Biosystems).

Quantitative PCR (qPCR)
qPCR reactions were performed using 250 nM of reverse and forward primers, 2 μl cDNA template (25 ng RNA/μl in the cDNA synthesis reaction and then diluted 1/10 prior to the PCR reaction) and the Roche LightCycler 400 SYBRGreen I Master (Roche) in a total volume of 10 μl. In the PCR reaction the hotstart Taq polymerase was activated for 10 min at 95°C, followed by 42 amplification cycles of 20 s denaturation at 95°C, 15 s annealing at primer-specific temperatures (Table S1 in File 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 expression levels were determined with the comparative delta threshold cycle (ΔΔCT) method. Relative expression levels of the target genes were normalized to the internal reference genes B2M, GAPDH and HPRT1 (PBMCs) or GAPDH and RPLP0 (adipose tissue) using the geNorm algorithm [34]. Briefly, the arithmetic mean of replicated Ct values for each gene is transformed to a relative quantity (setting the sample with the highest expression as calibrator to 1), using the formula $Q = E^{C_{target} - C_{calibrator}}$ (Q = quantity sample relative to calibrator sample; E = amplification efficiency). For normalization, the relative quantities were divided by the normalization factor being the geometric mean of the reference genes.

Data analysis
Linear regression analysis was performed using StatPlus* software, version 2009 (AnalystSoft, Alexandria, VA, USA). P-values were determined by analysis of variance (ANOVA). The ranking of vitamin D responsiveness was done under the assumption of a linear positive correlation between changes of the mRNA expression of CD14 or THBD and changes in the 25(OH)D3 serum concentrations. For each tissue and gene the samples were ranked symmetrically to the median of the sorted ratios of gene expression and 25(OH)D3 serum concentration changes. The mean of these four lists determined the final ranking, taking into account that for 24 participants no adipose tissue samples were available.

Results

Characteristics of the VitDmet cohort
With the aim to investigate possible effects of vitamin D3 supplementation on glucose metabolism and other features of the metabolic syndrome, a three-armed trial (placebo, 40 or 80 μg daily vitamin D3 supplementation over 5 months) had been designed. The study recruited 73 pre-diabetic (impaired fasting glucose or impaired glucose tolerance) human subjects from the region of Kuopio, Finland (63°N) [30]. The trial started in
October and finished at the end of winter (April), i.e. in a season of the year where in the Northern hemisphere at this latitude there is no natural UV-B source to induce vitamin D₃ synthesis in the skin of the participants.

In the present study, we focused on a sub-group of 71 participants of the above-described cohort, for which PBMC isolates were available from both the start and the end of the trial. For 47 of these 71 individuals also subcutaneous adipose tissue biopsies had been taken at both time points. The basic clinical and biochemical variables of the participants (Table 1) showed that neither body weight nor BMI changed significantly during the intervention in any of its arms. Importantly, in none of the three groups a significant change in serum calcium concentrations was observed, although one group obtained the rather high dose of 80 μg vitamin D₃/day. The baseline serum 25(OH)D₃ concentrations of 11 persons ranged between 35.9 and 73.6 nM (Tables 1 and Table S2 in File S2).

Consequently, based on the alternatively discussed threshold of 75 nM observed, although one group obtained the rather high dose of 80 μg vitamin D₃/day and by 44.9 nM in the group that received the highest supplementation dose of 80 μg (p<0.001 across the groups). Accordingly, serum PTH concentrations decreased in the groups with 40 and 80 μg daily vitamin D₃ doses as compared to the placebo group.

The analysis of the individual participants (Table S2 in File S2) showed that the serum 25(OH)D₃ concentrations of 11 persons decreased as much as 30.2 nM, mostly in the placebo group, while 25(OH)D₃ raised in the remaining participants by up to 87.2 nM. Taking the Institute of Medicine’s (IoM) recommended 25(OH)D₃ serum concentration of 50 nM as a reference [6], at the start of the trial 14 individuals (19.7%) had been vitamin D deficient, while at the end only 5 persons (7%) had low 25(OH)D₃ concentrations. In contrast, based on the alternatively discussed threshold of 75 nM [35], which was also applied as an inclusion criterion in the present study, at entry all participants (100%) had insufficient vitamin D concentrations, while after the intervention this applied only to 29 persons (40.0%), 10 of which were in the not-supplemented placebo control group. However, the variations in the serum 25(OH)D₃ concentrations were not fully consistent with the vitamin D₃ supplementation, which in part can be explained by the fact that all participants were allowed to continue their own vitamin D₃ supplementation with a lower dose (up to 20 μg/day). As we found no meaningful correlation of VDR target gene expression between the original study groups, for the following we ignored the information for the type of vitamin D₃ supplementation and took for each of the 71 participants only the relative changes in serum 25(OH)D₃ concentrations into account.

In summary, the gene expression analysis of this study is based on PBMC and adipose tissue samples, which were obtained from a cohort of 71 pre-diabetic elderly participants with a wide range of change in the 25(OH)D₃ serum concentrations after a 5-month vitamin D₃ supplementation during Finnish winter.

### Gene expression characteristics of the study participants

The PTH gene is a well-known down-regulated primary target of vitamin D and its receptor, while PTH protein signaling up-regulates CYP27B1 gene expression [36,37]. This inverse correlation between vitamin D and PTH concentrations is of major importance for appropriate bone mineralization [38]. In this study, we could confirm this inverse correlation, when plotting the relative change of PTH concentrations against the change of the serum 25(OH)D₃ concentrations (R² = 0.231, p = 0.00002; Fig S1 in File S1). This indicates that the overall vitamin D responsiveness of the 71 participants of the study is according to expectations.

Since the PTH gene is exclusively expressed in the parathyroid gland, i.e. in a tissue, which is not easily available for biopsies, it is not an appropriate choice for vitamin D gene expression studies. Therefore, we screened for more globally expressed primary VDR target genes. Our transcriptome- and genome-wide studies in THP-1 monocytes [21,39] suggested the genes CD14 and THBD to be well suited. In contrast to many other primary VDR target genes, CD14 and THBD are known for their prominent, long-lasting response to treatment with 1,25(OH)₂D₃. Moreover, both genes are regulated by a strong VDR binding site, which for CD14 is conserved between VDR ChIP-seq datasets of monocytes and colon cells (Fig. S2A in File S1) and for THBD even between monocytes, lymphoblastoids and colon cells (Fig. S2B in File S1). Interestingly, in a very recent study the same two genes were found

### Table 1. Main clinical and biochemical characteristics of the VitDmet study participants at baseline and their changes during the study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo group*, n = 22**</th>
<th>40 μg vitamin D₃/day group, n = 25</th>
<th>80 μg vitamin D₃/day group, n = 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum [25(OH)D₃] start (nM)</td>
<td>58.9+/−10.2</td>
<td>59.0+/−7.6</td>
<td>57.8+/−10.3</td>
</tr>
<tr>
<td>Delta serum [25(OH)D₃] (nM)</td>
<td>1.1 (−4.7; 6.9)</td>
<td>26.7 (20.0; 33.4)</td>
<td>44.8 (36.2; 53.4)</td>
</tr>
<tr>
<td>BMI start (kg/m²)</td>
<td>30.2+/−2.8</td>
<td>28.8+/−2.7</td>
<td>29.5+/−3.0</td>
</tr>
<tr>
<td>Delta BMI (kg/m²)</td>
<td>0.23 (−0.12; 0.58)</td>
<td>0.34 (0.13; 0.55)</td>
<td>0.33 (0.07; 0.53)</td>
</tr>
<tr>
<td>Serum [PTH] start (pg/ml)</td>
<td>44.6+/−18.2</td>
<td>41.5+/−9.5</td>
<td>43.8+/−11.1</td>
</tr>
<tr>
<td>Delta serum [PTH] (pg/ml)</td>
<td>4.7 (1.4; 8.0)</td>
<td>−0.5 (−3.5; 2.5)</td>
<td>−3.7 (−6.1; −1.3)</td>
</tr>
<tr>
<td>Serum [Ca] start (mM)</td>
<td>2.35+/−0.09</td>
<td>2.31+/−0.05</td>
<td>2.33+/−0.08</td>
</tr>
<tr>
<td>Delta serum [Ca] (mM)</td>
<td>−0.06 (−0.09; −0.03)</td>
<td>−0.04 (−0.07; −0.01)</td>
<td>−0.03 (−0.06; 0.00)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.4+/−5.7</td>
<td>66.2+/−5.5</td>
<td>66.4+/−4.3</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>3 (13.6%)/19 (86.4%)</td>
<td>4 (16%)/21 (84%)</td>
<td>3 (12%)/21 (88%)</td>
</tr>
</tbody>
</table>

*all participants were asked to keep their diet and other lifestyle habits unchanged during the study and were allowed to take up to 20 μg vitamin D₃/day.

**random assignment of the participants to the three groups.

Means and standard deviations for the baseline characteristics before intervention or mean parameter change with 95% confidence interval after the intervention are indicated for the three arms of the study. The detailed data of all 71 study participants are shown in Table S2 in File S2.

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in a comparison of a larger number of vitamin D transcriptomics studies (including our own) to be most significantly correlated with serum 25(OH)D3 concentrations of the Norwegian Women and Cancer Post-genome cohort [40]. Accordingly, we found that the relative mRNA expression of CD14 and THBD in PBMCs (normalized by three housekeeping genes) isolated from the 71 participants at the start of the study correlated significantly with the respective expression in cells taken at the end of the intervention (R² = 0.4123, p = 3.13.10⁻³⁰ and R² = 0.4185, p = 2.56.10⁻³⁸; Figs. 1A and B). This correlation came out to be even better, when the CD14 and THBD mRNA expression levels were further normalized by their respective VDR mRNA levels (R² = 0.6709, p = 2.49.10⁻¹⁰; and R² = 0.7428, p = 1.88.10⁻¹⁵, Figs. S3A and B in File S1).

For all 71 individuals the change of mRNA expression levels of CD14 and THBD positively correlated to each other (R² = 0.1479, p = 0.000099; Fig. 2A). Interestingly, an even far more significant correlation between the changes of CD14 and THBD mRNA expression levels was observed in adipose tissue samples obtained from 47 of the study participants (R² = 0.5978, p = 3.10⁻¹⁰; Fig. 2B).

Taken together, the expected negative correlation between serum concentrations of PTH and 25(OH)D₃ confirmed an overall normal physiological vitamin D response of the 71 study participants. The primary VDR target genes CD14 and THBD showed to be suitable transcriptomic biomarkers of vitamin D signaling both in PBMCs and adipocytes.

**CD14 and THBD mRNA expression changes efficiently classify study participants**

Based on the proven overall functionality of vitamin D signaling in the tissue samples of the study participants we assumed that an increase in serum 25(OH)D₃ concentrations would result in a respective increase in CD14 and THBD mRNA expression. However, neither with all 71 PBMCs donors nor with the subgroup of all 47 adipose tissue donors we could observe any significant correlation between the changes in 25(OH)D₃ serum concentrations and the mRNA expression changes of CD14 or THBD in the respective tissue samples (data not shown). Based on the assumption of a linear positive correlation between the changes of CD14 or THBD gene expression both in PBMCs and adipose tissue and changes of 25(OH)D₃ serum concentrations, we performed four separate rankings of the study participants, which were then combined to one ranking (Table S2 in File S2).

When we took only the top half of the ranking (35 persons), the plot of the changes in CD14 (Fig. 3A) or THBD (Fig. 3B) mRNA expression levels in PBMCs against the respective changes in 25(OH)D₃ concentrations provided statistically significant positive correlations (R² = 0.168, p = 0.01562 and R² = 0.243, p = 0.00324). We could confirm this observation with the adipose tissue samples, when restricting to the top half of the ranking in this tissue (23 persons). However, in adipose tissue the positive correlation between the changes in CD14 (R² = 0.1802, p = 0.02163; Fig. 3C) or THBD (R² = 0.1723, p = 0.08199; Fig. 3D) mRNA expression levels and their respective 25(OH)D₃ serum concentrations is less significant than in PBMCs. As a negative control for VDR target gene specificity, in neither of the two tissues the changes in FDR mRNA expression correlated with 25(OH)D₃ serum concentration changes (data not shown).

The categorization of the study participants by response to a change in their 25(OH)D₃ serum concentrations allows to observe correlations that are not visible when all participants are included in the analyses. For example, on the basis of all 71 participants there is no correlation between the change in serum concentrations of 25(OH)D₃ and the inflammatory marker IL6 [41] (data not shown). However, when we analyzed the segregated set of the 35 most vitamin D responsive samples, we found a significant negative correlation between changes in 25(OH)D₃ and IL6 (R² = 0.1352, p = 0.03; Fig. 4), as described earlier for monocytes [42].

In summary, the changes of CD14 or THBD mRNA expression levels in PBMCs and in adipose tissue samples in relation to 25(OH)D₃ serum concentrations suggest that it is possible to identify subjects who may benefit from vitamin D₃ supplementation, for example manifested by the down-regulation of the inflammatory marker serum IL6.

**Discussion**

During the last years a large number of population-based studies initiated a remarkable re-evaluation of the benefits of vitamin D for human health. This had been further catalyzed by the discussion on the recommendation of the IoM concerning the sufficient serum 25(OH)D₃ concentration of 50 nM [6]. Moreover, vitamin D belongs to the few nutritional compounds, which has, via its metabolite 1,25(OH)₂D₃, a direct effect on gene expression levels in PBMCs against the respective changes in 25(OH)D₃ serum concentrations provided statistically significant positive correlations (R² = 0.168, p = 0.01562 and R² = 0.243, p = 0.00324). We could confirm this observation with the adipose tissue samples, when restricting to the top half of the ranking in this tissue (23 persons). However, in adipose tissue the positive correlation between the changes in CD14 (R² = 0.1802, p = 0.02163; Fig. 3C) or THBD (R² = 0.1723, p = 0.08199; Fig. 3D) mRNA expression levels and their respective 25(OH)D₃ serum concentrations is less significant than in PBMCs. As a negative control for VDR target gene specificity, in neither of the two tissues the changes in FDR mRNA expression correlated with 25(OH)D₃ serum concentration changes (data not shown).

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In summary, the changes of CD14 or THBD mRNA expression levels in PBMCs and in adipose tissue samples in relation to 25(OH)D₃ serum concentrations suggest that it is possible to identify subjects who may benefit from vitamin D₃ supplementation, for example manifested by the down-regulation of the inflammatory marker serum IL6.

**CD14 and THBD as Transcriptomic Markers in VitDmet**

**Figure 1. The genes CD14 and THBD are suitable markers for vitamin D signaling in PBMCs.** For all 71 participants the mRNA expression in PBMCs of the genes CD14 (A) and THBD (B) at the start of the study correlate positively with their expression at the end. doi:10.1371/journal.pone.0071042.g001

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In conclusion, the results of our study suggest that the changes in serum 25(OH)D₃ concentrations correlated significantly with the respective mRNA expression changes of CD14 and THBD in PBMCs and adipose tissue. This finding is consistent with the assumption of a linear positive correlation between the changes of CD14 or THBD gene expression both in PBMCs and adipose tissue and changes of 25(OH)D₃ serum concentrations, which was confirmed with a ranking of the study participants. The results may have implications for the development of personalized nutrition strategies based on the current vitamin D status of the individual.
that some primary vitamin D target genes may serve as such biomarkers. We suggest that measuring the change in mRNA expression of the genes \textit{CD14} and \textit{THBD} in response to vitamin D$_3$ supplementation allows the evaluation of the responsiveness of the vitamin D signaling system of the human individual and provides a guideline if and how to adjust his/her serum vitamin D concentrations.

The PBMC and adipose tissue samples used in this study have the advantage of originating from a rather long vitamin D$_3$ intervention trial (5 months) with a placebo and a quite high dose of supplementation (up to 80 mg/day, i.e. 3,200 IU). The study was performed in the dark season of the year (Finnish winter) where there is no natural UV-B-induced endogenous production of vitamin D$_3$. This design resulted at the end of the study in i) a large range of serum 25(OH)D$_3$ concentrations between 27.5 and

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**Figure 2. The genes \textit{CD14} and \textit{THBD} correlate to each other in their expression.** In PBMCs (A) and adipose tissue (B) the ratios of the mRNA expression of \textit{CD14} at the end and the start of the study also positively correlate with the respective ratios of \textit{THBD} mRNA expression.

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**Figure 3. Changes of 25(OH)D$_3$ serum concentrations correlate with gene expression in PBMCs and adipocytes.** The study participants were ranked for their changes of \textit{CD14} and \textit{THBD} mRNA expression in PBMCs and adipocytes. For the top half of the PBMC donors (35 individuals, A and B) and also for the top half of the adipose tissue donors (23 individuals, C and D) changes of the mRNA expression of \textit{CD14} (A, C) and \textit{THBD} (B, D) from start to end of the study positively correlate with the respective change of 25(OH)D$_3$ serum concentrations.

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relationship between changes in 25(OH)D3 concentrations and supplementation. For the other half of the study group no most of the participants would not have needed a vitamin D from a 1.8-fold decrease to a 1.9-fold increase for Accordingly, the mRNA expression changes range in PBMCs experiment as it is the standard in mechanistic studies [39]. our approach is closer to the analysis of a typical ligand stimulation decrease of the baseline levels up to a 2.8-fold increase. In this way, were achieved by the VitDmet study, as a range from a 2.1-fold ranges of variation than THBD.

Although the ranges of the 25(OH)D3 serum concentration and VDR target gene changes during the intervention are in the same order, there is no statistically significant correlation between them, when all 71 study participants are studied. However, after ranking the study participants by the responsiveness of their CD14 and THBD expression to changes of 25(OH)D3 concentrations in both tested tissues, we found in the top half of the ranked participants a significant positive correlation. From the latter 35 individuals only 155.7 nM and ii) changes ranging from a decrease by 30.2 nM and an increase by 37.2 nM. In contrast to most other studies reported, we express these changes in relative and not in absolute terms, i.e. as a ratio and not as a difference. Therefore, we interpret the changes in serum 25(OH)D3 concentrations, which were achieved by the VitDmet study, as a range from a 2.1-fold decrease of the baseline levels up to a 2.8-fold increase. In this way, our approach is closer to the analysis of a typical ligand stimulation expression changes do not correlate with changes in 25(OH)D3 serum concentrations, the VDR gene shows similar ranges of variation than CD14 and THBD.

In conclusion, we suggest that the genes CD14 and THBD are suitable biomarkers for displaying the transcriptomic response of human tissues to vitamin D3 supplementation. However, only half of all investigated persons seem to respond in a way that we expected based on principles of vitamin D gene regulation to changes in their serum 25(OH)D3 concentrations, granting that from the response of their CD14 and THBD mRNA expressions straightforward conclusions on the benefits of a vitamin D3 supplementation can be obtained. This suggests a categorization of the study participants into those that are regular responders to vitamin D and others with an irregular response. The latter persons may have either some variation in their vitamin D signaling, which make more complex investigations necessary, or they simply have a sufficient vitamin D concentration, so that further supplementation is unnecessary.

Supporting Information

File S1
(PDF)

File S2
(XLSX)
Author Contributions

Conceived and designed the experiments: MU TPT JV SV CC. Performed the experiments: SS KP. Analyzed the data: CC SS. Wrote the paper: CC. Responsible for the dietary part of study management and implementation: SV Jv. Involved in the study methodology management and implementation: US VdM KP TN.

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