Genome-wide effects of vitamin D and the concept of the vitamin D response index

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I. Genome-wide view on vitamin D

II. The vitamin D response index
Vitamin D₃: A micronutrient entrained by light

There are two sources of vitamin D₃: diet and synthesis in the skin. The biologically active form of vitamin D₃, 1,25(OH)₂D₃, acts as a nuclear hormone by activating the nuclear receptor VDR.
The vitamin D receptor (VDR) is a transcription factor.

VDR belongs to the **nuclear receptor superfamily**, the member of which are activated by small lipophilic compounds that often derive directly from diet!

*Carlberg & Molnár, CJPP 2015*
Gene regulation by VDR requires accessible genomic DNA, i.e. open chromatin. In turn ligand-dependent actions of VDR (monitored by epigenome changes as shown next) result either in further opening of chromatin (i.e. in most cases in up-regulation of transcription of the respective gene) or in closing of chromatin (and respective down-regulation of transcription).
VDR binding in different cell types

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Cell type</th>
<th>Name</th>
<th>no ligand</th>
<th>VDR ligand</th>
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<tbody>
<tr>
<td>Ramagopalan</td>
<td>2010</td>
<td>B cells</td>
<td>GM10855</td>
<td>3,144</td>
<td>6,172</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GM10861</td>
<td>4,072</td>
<td>12,448</td>
</tr>
<tr>
<td>Heikkinen</td>
<td>2011</td>
<td>Monocytes</td>
<td>THP-1</td>
<td>613</td>
<td>774</td>
</tr>
<tr>
<td>Meyer</td>
<td>2012</td>
<td>Colon carcinoma</td>
<td>LS180</td>
<td>165</td>
<td>3,777</td>
</tr>
<tr>
<td>Ding</td>
<td>2013</td>
<td>Hepatic stellate</td>
<td>LX2</td>
<td>1,474</td>
<td>1,532</td>
</tr>
<tr>
<td>Tuoresmäki</td>
<td>2014</td>
<td>Macrophages</td>
<td>THP-1/LPS</td>
<td>529</td>
<td>955</td>
</tr>
</tbody>
</table>

MACS2 peak calling software using identical settings

In the presence of ligand, in average, **2.5-times more** genomic VDR binding sites are observed than in the absence of ligand. In total **23,409 non-overlapping VDR binding sites** are detected in 6 human ChIP-seq datasets.

LPS = polysaccharide; polarizes monocytes into M1-type macrophages

*Tuoresmäki et al., PLoS One 2014*
Scenario 1: VDR binds to core promoter regions

ABCD2 = ATP binding cassette subfamily D member 2: Transport protein, involved in adrenoleukodystrophy

Histone modifications
Transcription factor binding
Chromatin accessibility

THP-1 human macrophages

ABCD2 16.4-fold up

Nurminen et al., unpublished
Scenario 2: VDR binds to enhancer regions looping to core promoter regions

FBP1 = Fructose-1,6-bisphosphatase 1: Rate limiting enzyme in gluconeogenesis

VDR at enhancer and looping to TSS (core promoter)

FBP1 = Fructose-1,6-bisphosphatase 1: Rate limiting enzyme in gluconeogenesis

Nurminen et al., unpublished
Triplicate RNA-seq in THP-1 cells:

- 13,872 expressed genes (58.7% of all)
- within 24 h **587 genes** are significantly (p < 0.05) regulated by 1,25(OH)$_2$D$_3$
Early vitamin D target genes in human monocytes

217 genes that are significantly (p < 0.05) regulated by a stimulation of THP-1 cells for 2.5 h with 1,25(OH)₂D₃ are displayed (using a Manhattan plot).

Neme et al., JSBMB 2016
Late vitamin D target genes in human monocytes

587 genes that are significantly (p < 0.05) regulated by a stimulation of THP-1 cells for 24 h with 1,25(OH)₂D₃ are displayed (using a Manhattan plot).

Neme et al., JSBMB 2016
Latest version of the model of vitamin D signaling

1. Absence of ligand: VDR binds to a limited number of loci within accessible chromatin.
2. Presence of $1,25(OH)_2D_3$: The number of DNA-bound VDR molecules increases.
3. Pioneer factors (e.g., PU.1 in monocytes): VDR's access to genomic DNA further increases.
4. Chromatin accessibility: Local increase after VDR binding.
5. TAD anchors: CTCF sites upstream and downstream of prominent VDR binding sites increase in strength.
6. Vitamin D target genes: Located together with a prominent VDR site in a TAD flanked by vitamin D-sensitive CTCF sites.

TAD = topologically associated domain = chromatin loop

Carlberg, MCE 2017
I. Genome-wide view on vitamin D

II. The vitamin D response index
# Vitamin D parameters

## Vitamin D status

- Can vary a lot for a given individual
- Depends on sun exposure, nutrition and supplementation
- Established

## Vitamin D response index

- Stays constant over the lifetime of an individual
- Does *not* depend on nutrition, sun exposure or other environmental factors
- New concept, needs to be further explored

We need to know both the vitamin D status and the vitamin D response index, in order to obtain maximal health benefit from vitamin D.
The vitamin D status of a person is determined via the serum 25(OH)D level and can vary a lot over time.

Endocrine Society (US) recommendation

Institute of Medicine (US) recommendation

Monthly mean (±SD) of serum 25(OH)D concentrations among 1,136 older men and women from Finland.

Virtanen et al., EJN 2011
VitDmet study

daily 0, 40 or 80 µg vitamin D₃

<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>Δ 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>Δ 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>Δ 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>Δ 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

VitDmet (71 participants) has the same 3-arm study design as FIND (some 3,000 participants supplemented over 5 years).

Besides a rise in PTH levels no convincing effects of vitamin D, when applying the “delta” type of calculations.

Carlberg et al., PLoS One 2013
VitDmet: PBMC mRNA expression and serum protein levels

VitDmet:
71 elderly
(> 60 years)
pre-diabetic

daily 0, 40 or 80 µg vitamin D₃
qPCR from PBMCs
(T cells, B cells, monocytes)

In total we analyzed 24 vitamin D target genes and more than 100 clinical/biochemical parameters. All 24 genes and 12 of the clinical/biochemical parameters were vitamin D responsive!

CAMP: cathelicidin anti-microbial peptide; PTH: parathyroid hormone

Correlation analysis based on "fold change" instead of "delta"!

Saksa et al., JSBMB 2015
PTH is the expected center of the correlation network of the vitamin D response of the 71 prediabetic VitDmet subjects. Interestingly, the parameter \textit{fasting insulin} and the calculated values \textit{insulin sensitivity index} and \textit{HOMA-IR} are also dependent on the vitamin D responsiveness of the individuals and are centrally located in the correlation network (shaded grey).

\textit{Saksa et al., JSBMB 2015}
The responsiveness of an individual is proportional to his/her vitamin D index!

Vukic et al., PLoS One 2015
VitDmet and VitDbol: Different types vitamin D intervention trials

VitDmet
(NCT01479933):
71 elderly (> 60 years), pre-diabetic

daily 0, 40 or 80 µg vitamin D$_3$

VitDbol
(NCT02063334):
35 young (20-30 years), healthy

once 2,000 µg vitamin D$_3$ (up to 3 repeats in phase II)

PBMCs: qPCR, FAIRE-seq, RNA-seq

Epigenetic effects

The long time frame of the VitDmet study measures the transcriptional results of vitamin D-triggered epigenetic changes, while the VitDbol study measures in addition direct transcriptional effects.
VitDbol study: High, mid and low responders

(35 young, healthy subjects)

Also within a cohort of young healthy individuals there are some 25% low responders to vitamin D.
The vitamin D index concept

Step 1: Molecular basis of vitamin D action

Vitamin D₃ bolus $\rightarrow$ 25(OH)D₃ $\rightarrow$ 1,25(OH)₂D₃ $\rightarrow$ Epigenome changes (genomic VDR binding↑, histone markers, chromatin access↑)

Transcriptome changes (expression of ~400 vitamin D target genes changes within 24 h)

Data integration

Supplementation with personalized vitamin D doses

Individual vitamin D response index

High responders $\rightarrow$ Improvement of

Low responders

Bone mineralization (PTH)

Muscle function (grip strength/gait speed)

Immune function (cytokine expression)

Cellular growth (proliferation test)

Physiological effects of vitamin D on

Step 2: Clinical benefits of optimized vitamin D action
Hypotheses

When vitamin D intervention studies will be segregated for low responders, a more significant benefit of vitamin D supplementation should be observed.

Patients with a disease related to vitamin D deficiency, such as multiple sclerosis, will be to a higher percentage low vitamin D responders than healthy individuals.

=> when you know that you are a low vitamin D responder, you should take special care on your vitamin D status.
Measuring the vitamin D response index

Essential

Two blood samples of the same individual, e.g.,
- begin and end of winter (after a couple of months of supplementation)
- begin and end of summer (after a couple of months of no supplementation but normal outdoor activity)
- before and after a vitamin D bolus (over a few days, any time)

What to measure?
- Blood biochemistry (e.g., PTH)
- Gene expression of vitamin D target genes from PBMCs (e.g., CAMP or CD14)
- Chromatin changes in PBMCs in vitamin D responsive regions

Ranking in relation to a reference cohort
Acknowledgements

Dr. Sabine Seuter
Dr. Antonio Neme
Veijo Nurminen
Marina Alvarez
Maja Vukic
Noora Saksa
Julia Wilfinger
Dr. Jussi Ryynänen

Dr. Jyrki Virtanen
Dr. Ferdinand Molnár
Prof. Stine Marie Ulven
Prof. Moray Campbell
VitDbol: 35 healthy adults exposed to a vitamin D bolus

Individual changes in serum levels for $25$(OH)$D_3$, $1,25$(OH)$_2D_3$ and PTH at days 0, 1, 2 and 30.

Seuter et al., JSBMB 2016
**Summary**

There is some dispute on the desired **optimal vitamin D level and its recommended daily supplementation.**

Insight on the epigenome- and transcriptome-wide functions of vitamin D can be used for **determining of the optimal vitamin D status of human individuals.**

Based on vitamin D-dependent changes in gene expression of white blood cells as well as clinical/biochemical parameters, such as parathyroid hormone levels, **human individuals can be distinguished into high, mid and low responders to vitamin D.**

Long-term (VitDmet, NCT01479933) as well as short-term (VitDbol, NCT02063334) **vitamin D supplementation studies allow monitoring the vitamin D responsiveness of human individuals** and represent new types of human *in vivo* vitamin D investigations.

These observations led to the concept of a **personal vitamin D response index** that may be a better guideline for an optimized vitamin D supplementation than population-based recommendations.