Monocyte Innate Immune Response to Vitamin D

25(OH)D₃

1,25(OH)₂D₃

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Session MS40
Signaling: Vitamin D in Monocytes and Receptor Clustering in Mast Cells
Objectives

• Background on vitamin D and human health
• Overview of vitamin D metabolism and action, in particular, the role of serum vitamin D binding protein (DBP)
• Some findings on vitamin D and immune function and DBP
• Development of mathematical models
• Implications from mathematically analysis
Vitamin D Supplements
Historical: Childhood Rickets Due to Low Vitamin D
Public health significance:
Some (many?) may have vitamin D levels too low to actualize its full benefits.
Forms of vitamin D

- **U.V.B**
  - 7-dehydrocholesterol

- **Photolysis**
  - vitamin D₃
  - 25(OH)D₃
  - 1,25(OH)₂D₃
  - 1,24,25(OH)₃D₃

- **Enzymes**
  - 25-hydroxylase (CYP2R1)
  - 1α-hydroxylase (CYP27B1)
  - 24-hydroxylase (CYP24A1)

- **Functions**
  - Bone health
  - Immune regulation
  - Anti-proliferation

- **Metabolites**
  - 24,25(OH)₂D₃

**Steps**

1. **U.V.B** (UVB) photolysis of 7-dehydrocholesterol produces vitamin D₃.
2. Vitamin D₃ undergoes 25-hydroxylase (CYP2R1) to form 25(OH)D₃.
3. 25(OH)D₃ is converted to 1,25(OH)₂D₃ by 1α-hydroxylase (CYP27B1).
4. 1,25(OH)₂D₃ is reduced by 24-hydroxylase (CYP24A1) to form 1,24,25(OH)₃D₃.
5. 25(OH)D₃ is further metabolized to 24,25(OH)₂D₃ by 24-hydroxylase.
Vitamin D and Bacterial Killing
DBP polymorphisms

GC-1S  glu-al-a-thr-pro-thr

GC-1F  asp-al-a-thr-pro-thr

GC-2  asp-al-a-thr-pro-lys

1F > 1S > 2 affinity for 25D

Arnaud J, et al., Hum Genet. 1993 Sep;92(2):183-8
White GC-1S > GC-1F
Black GC-1F > GC-1S
White GC-2 > Black GC-2
USA Blacks
  GC-1F 70%
  GC-1S 16%
  GC-2  11%
USA Whites
  GC-1F 17%
  GC-1S 55%
  GC-2  26%

Since affinity for 25D: GC-1F > GC-1S > GC-2
Perhaps a basis for some differences between whites and blacks in vitamin D influenced health outcomes?
Questions

Did bound or free-ligand entry drive response?

How did DBP polymorphisms impact response?
Findings

Did bound or free-ligand entry drive response?
Free-ligand entry

How did DBP polymorphisms impact response?
Lower affinity DBP yielded higher response

*Vitamin D-Binding Protein Directs Monocyte Responses to 25-Hydroxy- and 1,25-Dihydroxyvitamin D*

Rene F. Chun, Anna L. Lauridsen, Lizabeth Suon, Lee A. Zella, J. Wesley Pike, Robert L. Modlin, Adrian R. Martineau, Robert J. Wilkinson, John Adams, and Martin Hewison

JCEM 95:3368 (2010)
How much free ligand?

<table>
<thead>
<tr>
<th></th>
<th>25D + DBP</th>
<th>25D/DBP</th>
<th>25D + ALB</th>
<th>25D/ALB</th>
<th>1,25D + DBP</th>
<th>1,25D/DBP</th>
<th>1,25D + ALB</th>
<th>1,25D/ALB</th>
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</thead>
<tbody>
<tr>
<td>Ka (M⁻¹)</td>
<td></td>
<td>7x10⁸</td>
<td></td>
<td>6x10⁵</td>
<td>4x10⁷</td>
<td></td>
<td>5.4x10⁴</td>
<td></td>
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</tbody>
</table>
How much free ligand?

Mathematical Theory of Cross-Reactive Radioimmunoassay and Ligand-Binding Systems at Equilibrium

HENRY FELDMAN, DAVID RODBARD, AND DANIEL LEVINE

National Institutes of Health, Bethesda, Maryland 20014

Received June 16, 1971

Laboratory of Biological Modeling
National Institute of Diabetes & Digestive & Kidney Diseases
Mathematical Analysis

Collaborators:
• Brad Peercy, Dept of Mathematics & Statistics, Univ of Maryland, Baltimore County
• Arthur Sherman, Lab of Biological Modeling, NIDDK, NIH
Mathematical Analysis

Expand existing mathematical model by adding ....
- Multiple genotypes for DBP (model #1)
- In vitro experimental data (model #2)

To ask ....
Using model #1
- How DBP genotype effects free levels of vitamin D ligands?

Using model #2
- Does the intracrine or endocrine mechanism account for vitamin D action in adherent monocytes?
# Model #1

## Variables and parameters

<table>
<thead>
<tr>
<th>Concentrations (user input):</th>
<th>Affinity (published):</th>
<th>Affinity differences (published):</th>
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</thead>
<tbody>
<tr>
<td>25D</td>
<td>DBP for 25D</td>
<td>GC1F/GC1F</td>
</tr>
<tr>
<td>1,25D</td>
<td>DBP for 1,25D</td>
<td>GC1F/GC1S</td>
</tr>
<tr>
<td>DBP</td>
<td>ALB for 25D</td>
<td>GC1F/GC2</td>
</tr>
<tr>
<td>ALB</td>
<td>ALB for 1,25D</td>
<td>GC1S/GC1S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GC1S/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GC2/2</td>
</tr>
</tbody>
</table>
General multiple buffer / multiple vitamin D model

Feldman, Rodbard & Levine, Analytical Biochemistry, 1972
Dunn, Annals of NY Academy of Sciences, 1988

\[ v_i \xrightarrow{+} b_j \xrightarrow{-} c_{ij} \]

where \( v_i \) are vitamin D isoforms \( i \in \{1, 2, \ldots, n\} \) and \( b_j \) are the buffers \( j \in \{1, 2, \ldots, m\} \). Law of Mass Action yields the differential equations.

\[
\frac{dv_i}{dt} = - \sum_j k_{i,j}^+ v_i b_j + \sum_j k_{i,j}^- c_{ij} \quad (1)
\]

\[
\frac{db_j}{dt} = - \sum_i k_{i,j}^+ v_i b_j + \sum_i k_{i,j}^- c_{ij} \quad (2)
\]

\[
\frac{dc_{ij}}{dt} = k_{i,j}^+ v_i b_j - k_{i,j}^- c_{ij} \quad (3)
\]
General multiple buffer / multiple vitamin D model

Conservation of vitamin D isoform and buffer yield, respectively

\[ v_i + \sum_j c_{ij} = V_{iT}, \quad b_j + \sum_i c_{ij} = B_j \]

In steady state, \( c_{ij} = \frac{v_i b_j}{K_{ij}} \) where \( K_{ij} = \frac{k_{ij}^-}{k_{ij}^+} \). So the free level of any buffer, \( b_j \), is

\[ b_j = \frac{B_j}{1 + \sum_i \frac{v_i}{K_{ij}}} \]

and consequently the free level of any vitamin D isoform, \( v_i \), is given by the solution to the \( n \) coupled algebraic equations

\[ v_i + v_i \sum_j \frac{1}{K_{ij} \left( 1 + \sum_i \frac{v_i}{K_{ij}} \right)} = V_{iT} \]
General multiple buffer / multiple vitamin D model

Presently, $v_i$ are vitamin D isoforms $i \in \{25D, 1,25D\}$ and $b_j$ are the buffers $j \in \{DBP, Albumin\}$. This yields the system of two algebraic equations for free 25D and 1,25D

\[
v_1 \left(1 + \frac{K_1 P}{1 + K_1 v_1 + K_2 v_2} + \frac{K_3 B}{1 + K_3 v_1 + K_4 v_2}\right) = V_{1T}
\]

\[
v_2 \left(1 + \frac{K_2 P}{1 + K_1 v_1 + K_2 v_2} + \frac{K_4 B}{1 + K_3 v_1 + K_4 v_2}\right) = V_{2T}
\]

where $P = [DBP]$ and $B = [Albumin]$. The $K_{i+2(j-1)}$'s here are $1/K_{ij}$'s above.
Presently, \( v_i \) are vitamin D isoforms \( i \in \{25D, 1,25D\} \) and \( b_j \) are the buffers \( j \in \{DBP_a, DBP_b, Albumin\} \) and \( a, b \in \{GC1F, GC1S, GC2\} \) are alleles of the DBP. This yields the expanded system of two algebraic equations

\[
v_1 \left(1 + \frac{K_1 P_a}{1 + K_1 v_1 + K_2 v_2} \right) + \frac{K_3 B}{1 + K_3 v_1 + K_4 v_2} + \frac{K_5 P_b}{1 + K_5 v_1 + K_6 v_2} = V_{1T}
\]

\[
v_2 \left(1 + \frac{K_2 P_a}{1 + K_1 v_1 + K_2 v_2} \right) + \frac{K_4 B}{1 + K_3 v_1 + K_4 v_2} + \frac{K_6 P_b}{1 + K_5 v_1 + K_6 v_2} = V_{2T}
\]
Free Vitamin Levels Based on Genotypic Variation in DBP

In vitro (5% serum)     In vivo

A  
B

C

D

**Free Vitamin Levels Based on Genotypic Variation in DBP**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>25OHD (nM)</th>
<th>25OHD (nM)</th>
<th>25OHD (nM)</th>
<th>25OHD (nM)</th>
<th>25OHD (nM)</th>
<th>25OHD (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC1F/1F</td>
<td>25</td>
<td>0.006</td>
<td>50</td>
<td>0.013</td>
<td>100</td>
<td>0.025</td>
</tr>
<tr>
<td>GC1F/1S</td>
<td>25</td>
<td>0.008</td>
<td>50</td>
<td>0.016</td>
<td>100</td>
<td>0.032</td>
</tr>
<tr>
<td>GC1F/2</td>
<td>25</td>
<td>0.010</td>
<td>50</td>
<td>0.019</td>
<td>100</td>
<td>0.039</td>
</tr>
<tr>
<td>GC1S/1S</td>
<td>25</td>
<td>0.011</td>
<td>50</td>
<td>0.021</td>
<td>100</td>
<td>0.043</td>
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<tr>
<td>GC1S/2</td>
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<td>50</td>
<td>0.028</td>
<td>100</td>
<td>0.055</td>
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<tr>
<td>GC2/2</td>
<td>25</td>
<td>0.018</td>
<td>50</td>
<td>0.037</td>
<td>100</td>
<td>0.074</td>
</tr>
</tbody>
</table>

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“**Sufficiency**” as described in Nov 2010 report by Institute of Medicine (IOM): 20 nanograms per milliliter (50 nM) serum 25D is needed for good bone health for practically all individuals.
Model #2

v1 = 25D
v2 = 1,25D

extracellular

intracellular

Albumin:v1

DBP:v1

VDR:v1

CYP27B1

CAMP

Kₐ=700µM⁻¹

Kₐ=5µM⁻¹

Kₐ=10⁴µM⁻¹

Kₐ=0.6µM⁻¹

Kₐ=40µM⁻¹

Kₐ=0.054µM⁻¹

VDR:v2

DBP:v2

CAMP

CAMP DNA

Kₐ=1µM

Kₐ=1µM

Kₐ=0.054µM⁻¹

Kₐ=10⁴µM⁻¹
Parameters and variables for model #2

Some knowledge exists …
From model #1
• Affinity of DBP for 25D & 1,25D
• Affinity differences in DBP genotypes
• Amounts of DBP in differing genotypes
• Affinity of ALB for 25D & 1,25D

Needed for model #2
• Affinity of VDR for 1,25D (much less known for 25D)
• Amount of VDR in cells (has been estimated in some cell types)
• Permeability of cells for 1,25D (none for 25D)
• Affinity of CYP27B1 for 25D (reconstitution studies)
• Rate of conversion to 1,25D by CYP27B1 (reconstitution studies)

Guesstimated …
• Amount of CYP27B1
• Amount of CYP27B1 and VDR in activated immune cell
• Affinity of 25D/VDR binding to VDRE
• Affinity of 1,25D/VDR binding to VDRE (EMSA data exists)
• Effectiveness of 25D/VDR/VDRE complex on transcription of CAMP
• Effectiveness of 1,25D/VDR/VDRE complex on transcription CAMP
• Extracellular volume >> intracellular volume
Blood steady state

When $\gamma \gg 1$, the extracellular vitamin D level is set by the buffers. The intracellular level is then set by the activation level of the enzyme balanced with the input of vitamin D from the exterior.

$$y_1(v_1^i) = \frac{Y_T(v_1^i)^n}{(v_1^i)^n + K_m^n}$$

where $K_m = (k_b + k_{cat})/k_f$ is the standard Michaelis-Menten constant. This leaves us with the nonlinear algebraic equation to solve in steady state for $v_1^i$

$$0 = -nk_{cat}y_1(v_1^i) + d(v_1^0 - v_1^i)$$

which we can then use to get $v_2^i$,

$$v_2^i = v_2^0 + \frac{n}{d} k_{cat}y_1(v_1^i)$$
VDR/Ligand Interactions

25D $\leftrightarrow$ VDR/25D

$25D \leftrightarrow VDR/25D$

$$r_1 = \frac{K_r^{pp} v_1^{emm} R_T}{K_r^{pp} v_1^{emm} + K_r^{mm} v_2^{cpp} + K_r^{mm} K_r^{pp}},$$

1,25D $\leftrightarrow$ VDR/1,25D

$$1,25D \leftrightarrow VDR/1,25D$$

$$r_2 = \frac{K_r^{mm} v_2^{cpp} R_T}{K_r^{pp} v_1^{emm} + K_r^{mm} v_2^{cpp} + K_r^{mm} K_r^{pp}},$$

$r_2 > r_1$
Active Transcription Complexes

\[ o_1 = \frac{K_{cc2}^p r_1^m}{K_{cc1}^m r_1^p + K_{cc2}^p r_1^m + K_{cc1}^m K_{cc2}^p} \quad , \quad o_2 = \frac{K_{cc1}^m r_2^p}{K_{cc1}^m r_2^p + K_{cc2}^p r_2^m + K_{cc1}^m K_{cc2}^p} \]

\( o_1 \) = active complex containing 25D

\( o_2 \) = active complex containing 1,25D

\( o_2 > o_1 \)
CAMP production

\[
\begin{align*}
  r_1 &= \frac{K_{r2}^{pp} v_1^{\text{c}} \cdot R_T}{K_{r2}^{pp} v_1^{\text{c}} + K_{r1}^{\text{m}} v_2^{\text{c}} + K_{r1}^{\text{m}} K_{r2}^{pp}}, \\
  r_2 &= \frac{K_1^{\text{mm}} v_2^{\text{c}pp} R_T}{K_{r2}^{pp} v_1^{\text{c}} + K_{r1}^{\text{m}} v_2^{\text{c}} + K_{r1}^{\text{m}} K_{r2}^{pp}}, \\
  o_1 &= \frac{K^{p}_{cc2} r_1^{m}}{K^{m}_{cc1} r_2^{p} + K^{p}_{cc2} r_1^{m} + K^{m}_{cc1} K^{p}_{cc2}}, \\
  o_2 &= \frac{K^{m}_{cc1} r_2^{p}}{K^{m}_{cc1} r_2^{p} + K^{p}_{cc2} r_1^{m} + K^{m}_{cc1} K^{p}_{cc2}}.
\end{align*}
\]

\[
CAMP = \eta \left( o_1 + o_2 \right) + CAMP_0
\]

…. solve computationally and then express graphically …. 

\[
\Delta\Delta CT = \log_2(CAMP/CAMP_0)
\]
In vitro (5% serum) data and model fit

Then adjust parameters to project in vivo responses …
## Intracrine vs. Endocrine?

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Both mechanisms</th>
<th>Intracrine mechanism</th>
<th>Endocrine mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 nM 25OHD</td>
<td>50 nM 25OHD</td>
<td>0 nM 25OHD</td>
</tr>
<tr>
<td>Subject</td>
<td>0.1 nM 1,25(OH)₂D</td>
<td>0 nM 1,25(OH)₂D</td>
<td>0.1 nM 1,25(OH)₂D</td>
</tr>
<tr>
<td></td>
<td>Basal</td>
<td>Activated</td>
<td>Basal</td>
</tr>
<tr>
<td>GC1F/1F</td>
<td>0.010</td>
<td>0.011</td>
<td>0.010</td>
</tr>
<tr>
<td>GC1F/1S</td>
<td>0.010</td>
<td>0.012</td>
<td>0.010</td>
</tr>
<tr>
<td>GC1F/2</td>
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<td>0.015</td>
<td>0.010</td>
</tr>
<tr>
<td>GC1S/1S</td>
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<td>0.019</td>
<td>0.010</td>
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<tr>
<td>GC1S/2</td>
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<td>0.032</td>
<td>0.010</td>
</tr>
<tr>
<td>GC2/2</td>
<td>0.010</td>
<td>0.072</td>
<td>0.010</td>
</tr>
</tbody>
</table>

doi:10.1371/journal.pone.0030773.t006

**Basal** = 1x VDR, 1x CYP27B1

**Activated** = 5x VDR, 10x CYP27B1
Summary

- Local production of 1,25D from 25D is a plausible mechanism to account for vitamin D action in immune cells.

- DBP genotype, DBP amount and total 25D interact to determine free 25D levels which impacts vitamin D action.

- Thus, one fixed amount of total 25D to define sufficiency may be an inadequate clinical guideline.