Current Knowledge of Vitamin D Metabolism and Function

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RECENT ADVANCES IN VITAMIN D RESEARCH

- EPIDEMIOLOGICAL STUDIES OF VITAMIN D DEFICIENCY
  - SUGGEST ASSOCIATION TO MANY DISEASE STATES

- VDR GENE EXPRESSION STUDIES
  - SUGGEST # OF VIT D-DEPENDENT GENES = 300-800

- CLONING OF THE 1α-HYDROXYLASE (CYP27B1)
  - WIDESPREAD DISTRIBUTION NOT JUST KIDNEY
Vitamin D Metabolism & Function

Objectives:

• Review current knowledge of Vitamin D Metabolism
  - New information about the cytochrome P450s involved
  - Concept of extra-renal 1α-hydroxylase

• Review the classical and non-classical roles of Vitamin D
  - Importance of calcitriol in VDR-mediated gene expression

• Implications of vitamin D renaissance for physicians
  - Serum 25-OH-D assay as a Biomarker for vitamin D status
  - Vitamin D Deficiency may underlie several major diseases
  - Vitamin D Supplementation
Metabolism of Vitamin D₃

Vitamin D₃ → 25-OH-D₃ → 1α,25-(OH)₂D₃

CYP27A1, CYP2R1 Liver → OH

CYP27B1 Kidney → OH

Similar pathway exists for vitamin D₂
Metabolism of Vitamin D$_3$

Vitamin D$_3$ → 25-OH-D$_3$

CYP27A1
CYP2R1
CYP3A4
Perfused Liver reveals two 25-Hydroxylases

Table 1. Two modes of the 25-hydroxylation reaction of vitamin D₃ in rat liver.

<table>
<thead>
<tr>
<th>Mode of Reaction</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent Km</td>
<td>$5.6 \times 10^{-6}$ M</td>
<td>$5.0 \times 10^{-6}$ M</td>
</tr>
<tr>
<td>Substrate specificity</td>
<td>Specific</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Amounts of Enzyme</td>
<td>limited</td>
<td>large</td>
</tr>
<tr>
<td>(Binding capacity)</td>
<td>&lt; 1.3 nmoles</td>
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</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Velocity</td>
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CYP2R1

• LIVER MICROsomal Vitamin D 25-Hydroxylase

• Substrates Include Vitamins D$_3$ & D$_2$ & Anticancer Prodrugs (eg 1α-OH-D$_2$)

• Mutation in hCYP2R1 at L99P Causes Rickets

• Works at Nanomolar Substrate & Probably the Physiologically Relevant Vit.D-25-Hydroxylase

• SGC (Toronto) Crystallised a Functional Human CYP2R1 with Vitamin D$_3$ in Active Site
25-Hydroxylation of 1α-OH-D₂ by Mouse cyp2R1

Mouse CYP2R1 transfection using V79-4 cells with 3H 1aD₂ as substrate.

1α-OH-D₂

1α,25-(OH)₂D₂
Perfused Liver reveals two 25-Hydroxylases

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<th>CYP27A1</th>
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<tr>
<td>Apparent Km</td>
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Metabolism of Vitamin D$_3$

Vitamin D$_3$ → 25-OH-D$_3$ → 1α,25-(OH)$_2$D$_3$

Liver: CYP27A1, CYP2R1
Kidney: CYP27B1

Calcitriol

Similar pathway exists for vitamin D$_2$
CYP27B1

• KIDNEY MITOCHONDRIAL 1α-HYDROXYLASE

• SUBSTRATES INCLUDE 25-OH-D₃ & 25-OH-D₂

• MUTATIONS IN hCYP27B1 CAUSE VDDR-RICKETS TYPE 1

• PROBES & ANTIBODIES REVEAL PRESENCE OF CYP27B1 IN EXTRA-RENAAL TISSUES eg SKIN, MONOCYTE

• REGULATION DIFFERENT IN DIFFERENT TISSUES:
  A) KIDNEY CYP27B1—PTH & FGF-23; Ca & PO₄
  B) EXTRA-RENAAL CYP27B1 -- CYTOKINES
CYP27B1

- KIDNEY MITOCHONDRIAL 1α-HYDROXYLASE
- SUBSTRATES INCLUDE 25-OH-D$_3$ & 25-OH-D$_2$
- MUTATIONS IN hCYP27B1 CAUSE VDDR-RICKETS TYPE I
- PROBES & ANTIBODIES REVEAL PRESENCE OF CYP27B1 IN EXTRA-RENAL TISSUES eg SKIN, MONOCYTES
- REGULATION DIFFERENT IN DIFFERENT TISSUES:
  A) KIDNEY CYP27B1–PTH & FGF-23; Ca & PO$_4$
  B) EXTRA-RENAL CYP27B1 -- CYTOKINES
Plasma Calcium Homeostasis

CT = calcitonin
ECF = extracellular fluid
PTG = Parathyroid gland
CaSR = Calcium Sensing Receptor
Roles of FGF23
1) Increase $\text{PO}_4$ excretion
2) Decrease CYP27B1
3) Increase CYP24A1 & NET INCREASE IN D CATABOLISM
CYP24A1

- MITOCHONDRIAL 25-OH-D 24-HYROXYLASE IN KIDNEY AND ALL TARGET CELLS

- SUBSTRATES INCLUDE 25-OH-D₃ & 1α,25-(OH)₂D₃ & CYP24 IS A MULTICATALYTIC ENZYME

- INDUCED BY 1α,25-(OH)₂D₃ & FGF-23 IN TARGET CELLS

- CYP24A1 KNOCKOUT MOUSE SHOWS 50% LETHALITY

- MAJOR ROLE IS CATABOLIC AND EXISTS TO ATTENUATE ACTION OF CALCITRIOL INSIDE TARGET CELLS
Metabolism of Vitamin D

Vitamin D₃

Liver

CYP27A1
CYP27B1
CYP3A4

25-OH-D₃

Kidney

CYP27B1

1α,25-(OH)₂D₃

CYP24

24,25-(OH)₂D₃

Calcitriol Acid

1α,24,25-(OH)₃D₃

BILE

1α,25-(OH)₂D₃-26,23-lactone

24,25-(OH)₂D₃

CYP24 IS INDUCED BY ITS SUBSTRATE

1α,25-(OH)₂D₃ \rightarrow 24\text{-hydroxylase CYP24} \rightarrow \text{Calcitroic Acid}

Transcriptional up-regulation

- Kidney
  - CYP24 mRNA
  - β-ACTIN mRNA
- Liver

+
$[^3]H \alpha,25\text{-}(\text{OH})_2\text{D}_3$ in Blood of Wild type and Cyp24-XO Mice

Masuda et al Endocrinology, 2005
Vitamin D Target Cell

Blood Vessel

1α,25-(OH)₂D₃

DBP

Calcitriolic Acid

Nucleus

RXR/VDR

RNA Polymerase

VDRE

Vitamin D-dependent gene

CYP24
Vitamin D – Endocrine & Intracrine System

Vitamin D3
25-OH-D3
1α,25-(OH)2D3
Calcitriol
DBP
Megalin/cubulin

Liver

Nucleus
CYP27A1
CYP2R1

Kidney

Nucleus
CYP27B1
megalin/cubulin

Extra-renal 1α-hydroxylating target cells
Vitamin D-dependent genes
p21
mRNA
CYP24A1
RXR/VDR
VDRE
Cytokines
Calbindins

Normal target cells
Vitamin D-dependent genes
p21
Osteopontin
Calbindins

Vitamin D Metabolism & Function

Objectives:

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  - New information about the cytochrome P450s involved
  - Concept of extra-renal 1α-hydroxylase

• Review the classical and non-classical roles of Vitamin D
  - Importance of calcitriol in VDR-mediated gene expression

• Implications of vitamin D renaissance for physicians
  - Serum 25-OH-D assay as a Biomarker for vitamin D status
  - Vitamin D Deficiency may underlie several major diseases
  - Vitamin D Supplementation
Mechanism of Action of Vitamin D

1,25(OH)₂D₃ ↔ DBP

24-OHase Initiated Catabolic Cascade

Calcitroic Acid

VDR

D₃

24-OHase

GGGTGA ACG GGGGCA

VDRE

Transcription Regulation

↑/↓ mRNA levels

↑ CaBP
↑ ECaC
↑ 24(OH)ase
↑ Osteocalcin
↑ Osteopontin
↑ RANKL
↑ Alk Pase
↑ p21
↑ p27
↑ PSA
↓ Collagen
↓ PTH

Transepithelial Calcium Transport
Degradation
Bone Metabolism
Intracellular Calcium Modulation
Regulation of Cell Proliferation & Differentiation
Microarray Analysis of Calcitriol Treatment Breast Cancer Cells

MCF-7
ERα(+)

19 Cell Cycle/Apoptosis
6 Cell Adhesion
19 Cell Cycle/Apoptosis
3 DNA Repair
7 Growth/Immune Mod.
2 Steroid Receptors
2 Oncogenes
8 Others

MB231
ERα(-)

2 Cell Adhesion
5 Cell Cycle/Apoptosis
2 Growth/Immune Mod.
1 Steroid Receptors
1 Oncogene
3 Trans Factors/Kinases
3 Cell Adhesion
2 Cell Cycle/Apoptosis
5 Growth/Immune Mod.
3 Cell Adhesion
2 Cell Cycle/Apoptosis
5 Growth/Immune Mod.
3 Cell Adhesion
2 Cell Cycle/Apoptosis
5 Growth/Immune Mod.

49
16
49
16

Rb2
TGFβ2
RAB5A
Integrin αV
Thioredoxin Red.
CYP24
VDR
BRCA2

2000 Gene Probes

Calcitriol blocks prostaglandin pathways
Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes\textsuperscript{1−3} \textit{Am J Clin Nutr} 2006;84:18–28.

Heike A Bischoff-Ferrari, Edward Giovannucci, Walter C Willett, Thomas Dietrich, and Bess Dawson-Hughes
Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial

Joan M Lappe, Dianne Travers-Gustafson, K Michael Davies, Robert R Recker, and Robert P Heaney

1180 WOMEN FOLLOWED 5 YR
Ca = 1400 mg/d
VITAMIN D = 1100 IU/d

BLOOD 25-OH-D level
Ca+D = 72 \rightarrow 96 \text{ nmol/L}
Ca ONLY = 71 \text{ nmol/L}
PLACEBO = 71 \text{ nmol/L}
25(OH)D
Major Circulating Metabolite

Keratinocytes

Kidney

Macrophages

Colon
Prostate
Breast etc.

1,25(OH)_2D
Biologically Active

Calcium and Phosphorus Homeostasis
Bone Health

Growth & Regulation
- Antiproliferation
- Prodifferentiation
- Apoptotic
- Anti-angiogenic
- Prostate, Colon, Breast Cancers etc.

Immunomodulatory Effects
- Multiple Sclerosis
- Type I Diabetes (via β-islet cell destruction)
- Psoriasis
- Rheumatoid Arthritis
- Inflammatory Bowel Disease
- Periodontal Disease

Cardiovascular Effects
- Renin-Angiotensin Regulation
- Decreased Risk for:
  - Hypertension
  - Type II Diabetes (via stimulation of pancreatic insulin production)
  - Heart Failure

Neuromuscular Effects
- Muscle Mass
- Muscle Strength
- Better Balance
Antimicrobial response
Chemotaxis
Phagocytosis
Cathelicidin
Defensin beta4
Reactive oxygen species

25(OH)D₃

1αOHase

Mon/Mφ

1,25(OH)₂D₃

IL-1
IL-6
TNF-α

IL-12
IL-6
TNF-α
IL-23

IL-10
CCL22

CD80/86

MHC II

CD40

TCR

CD40L

CD4+T

25(OH)D₃

CD4+T

Th17

Th1

Th1

Th1

Th17

Th17

Th2

Th2

Th2

Th2

Treg

Treg

IL-4
IL-5
IL-13

IL-10

TGF-β

CHANTAL MATHIEU, U OF LEUVEN, 2009
Toll-Like Receptor Triggering of a Vitamin D–Mediated Human Antimicrobial Response

Philip T. Liu,1,2* Steffen Stenger,4* Huiying Li,3 Linda Wenzel,4 Belinda H. Tan,1,2 Stephan R. Krutzik,2 Maria Teresa Ochoa,2 Jürgen Schauber,5 Kent Wu,1 Christoph Meinken,4 Diane L. Kamen,6 Manfred Wagner,7 Robert Bals,8 Andreas Steinmeyer,9 Ulrich Zügel,10 Richard L. Gallo,5 David Eisenberg,3 Martin Hewison,11 Bruce W. Hollis,12 John S. Adams,11 Barry R. Bloom,13 Robert L. Modlin1,2†

In innate immune responses, activation of Toll-like receptors (TLRs) triggers direct antimicrobial activity against intracellular bacteria, which in murine, but not human, monocytes and macrophages is mediated principally by nitric oxide. We report here that TLR activation of human macrophages up-regulated expression of the vitamin D receptor and the vitamin D-1–hydroxylase genes, leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular Mycobacterium tuberculosis. We also observed that sera from African-American individuals, known to have increased susceptibility to tuberculosis, had low 25-hydroxyvitamin D and were inefficient in supporting cathelicidin messenger RNA induction. These data support a link between TLRs and vitamin D–mediated innate immunity and suggest that differences in ability of human populations to produce vitamin D may contribute to susceptibility to microbial infection.
Evidence of a role for $1,25-(OH)_2D$ in Skin
1,25-(OH)$_2$D & Renin-Angiotensin System

1,25(OH)$_2$D functions directly and negatively as a novel endocrine regulator of the renin-angiotensin system in vivo and in vitro

- 1,25(OH)$_2$D markedly suppresses renin transcription by VDR-mediated mechanism in cell cultures and animal models
- VDR-Knockout animal studies demonstrate the importance of maintaining normal serum levels of 1,25(OH)$_2$D for proper homeostasis of calcium, electrolytes, volume, and blood pressure

Plasma 25-Hydroxyvitamin D Levels and Risk of Incident Hypertension

John P. Forman, Edward Giovannucci, Michelle D. Holmes, Heike A. Bischoff-Ferrari, Shelley S. Tworoger, Walter C. Willett, Gary C. Curhan

Abstract—Hydroxylation of 25(OH)D to 1,25-dihydroxyvitamin D and signaling through the vitamin D receptor occur in various tissues not traditionally involved in calcium homeostasis. Laboratory studies indicate that 1,25-dihydroxyvitamin D suppresses renin expression and vascular smooth muscle cell proliferation; clinical studies demonstrate an inverse association between ultraviolet radiation, a surrogate marker for vitamin D synthesis, and blood pressure. We prospectively studied the independent association between measured plasma 25-hydroxyvitamin D [25(OH)D] levels and risk of incident hypertension and also the association between predicted plasma 25(OH)D levels and risk of incident hypertension. Two prospective cohort studies including 613 men from the Health Professionals’ Follow-Up Study and 1198 women from the Nurses’ Health Study with measured 25(OH)D levels were followed for 4 to 8 years. In addition, 2 prospective cohort studies including 38,388 men and 77,531 women with predicted 25(OH)D levels were followed for 16 to 18 years. During 4 years of follow-up, the multivariable relative risk of incident hypertension among men whose measured plasma 25(OH)D levels were <15 ng/mL (ie, vitamin D deficiency) compared with those whose levels were ≥30 ng/mL was 6.13 (95% confidence interval [CI]: 1.00 to 37.8). Among women, the same comparison yielded a relative risk of 2.67 (95% CI: 1.05 to 6.79). The pooled relative risk combining men and women with measured 25(OH)D levels using the random-effects model was 3.18 (95% CI: 1.39 to 7.29). Using predicted 25(OH)D levels in the larger cohorts, the multivariable relative risks comparing the lowest to highest deciles were 2.31 (95% CI: 2.03 to 2.63) in men and 1.57 (95% CI: 1.44 to 1.72) in women. Plasma 25(OH)D levels are inversely associated with risk of incident hypertension. (Hypertension. 2007;49:1063-1069.)

Key Words: vitamins ■ epidemiology ■ hypertension ■ risk factors ■ human
1,25-(OH)_2D & Muscle Differentiation

Muscle Fiber Diameter

Protein Expression

VDR -/-

VDR +/-

VDR -/-

VDR +/-
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  - Vitamin D Supplementation
Prevention and treatment of vitamin D insufficiency and vitamin D deficiency in CKD patients.

**SUGGESTED THRESHOLD = 30 ng/mL or 75 nmol/L**
Seasonal variance of 25-(OH) vitamin D in the general population of Estonia, a Northern European country at Latitude 59° N
Mart Kull Jr*1,2, Riina Kallikorm1,2, Anu Tamm2 and Margus Lember1,2

BMC Public Health 2009, 9:22
CORRECTION OF 25-OH-D INSUFFICIENCY

• INCREASED UV EXPOSURE
  - OPPOSED BY DERMATOLOGISTS

• FOOD FORTIFICATION
  - MOST FOODS POOR IN VITAMIN D (except SALMON)
  - FORTIFICATION VARIES WITH STATE--- Dairy Products
  - MILK INTOLERANCE & DOSAGE ISSUES

• ORAL VITAMIN D SUPPLEMENTS
  - PRESCRIPTION-VIT. D$_2$ in US (Drisdol)- 50,000 IU/dose
  - OTC VITAMIN PILLS-CURRENT DRI- 400-800 IU/day
  - OTC “DIETARY D$_3$ SUPPLEMENTS” IN USE- 1000-2000 IU
  - NATIONAL ACADEMY OF SCIENCE- NEW DRI-FALL 2010
Exposure to UVB radiation in sunlight is the single greatest source of vitamin D for most individuals, so location and season affect a population's risk of deficiency. For periods of the year known as vitamin D winter, UVB intensity is too weak at some latitudes even to induce vitamin D synthesis in the skin. Because ozone blocks UVB rays, the rays are most intense nearest the equator, where sunlight travels the least distance through the earth's atmosphere, and vitamin D synthesis is possible year-round. An increasing angle of penetration at higher latitudes weakens UVB intensity until it is insufficient, especially during winter, for making vitamin D.
Sources of Vitamin D

Sun (April to October)

Diet (Dairy & Fish)
Summary

• The Vitamin D Metabolic Machinery is series of CYPs operating in a well-integrated Endocrine-Intracrine system

• \(1\alpha,25-(OH)_2\)D has many varied classical and non-classical roles around the body

• Emergence of extra-renal \(1\alpha\)-hydroxylase emphasizes the value of serum 25-OH-D assay as a tool to monitor vitamin D status

• Much interest in determining the underlying importance of vitamin D deficiency/insufficiency in various common diseases

• Vitamin D Supplementation should be considered
Reviews at <gj1@queensu.ca>


