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

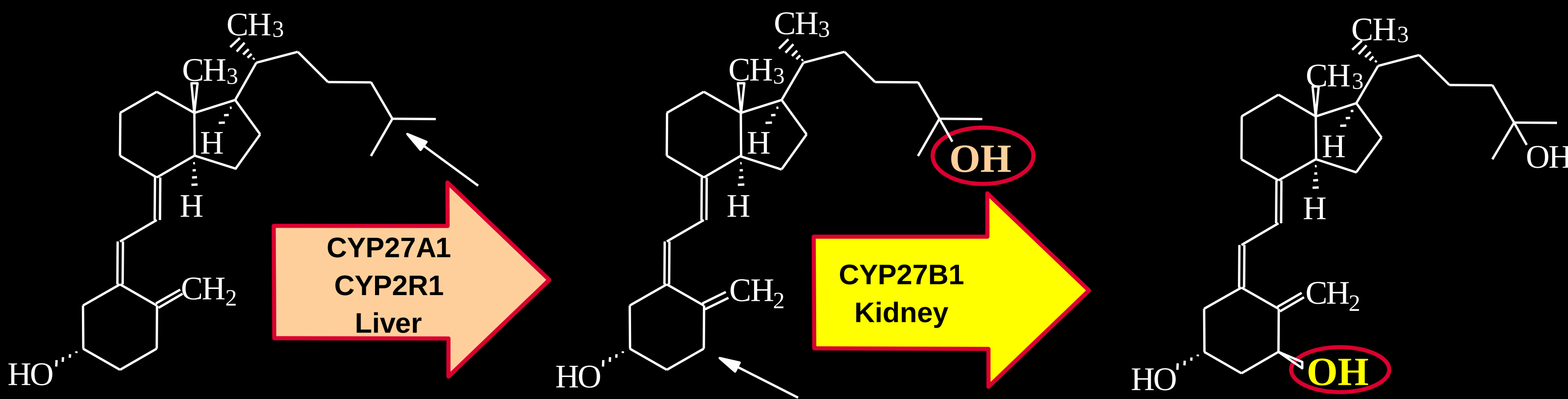
- CLONING OF THE 1α-HYDROXYLASE (CYP27B1)
  - WIDESPREAD DISTRIBUTION NOT JUST KIDNEY

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 D_3



Vitamin D_3

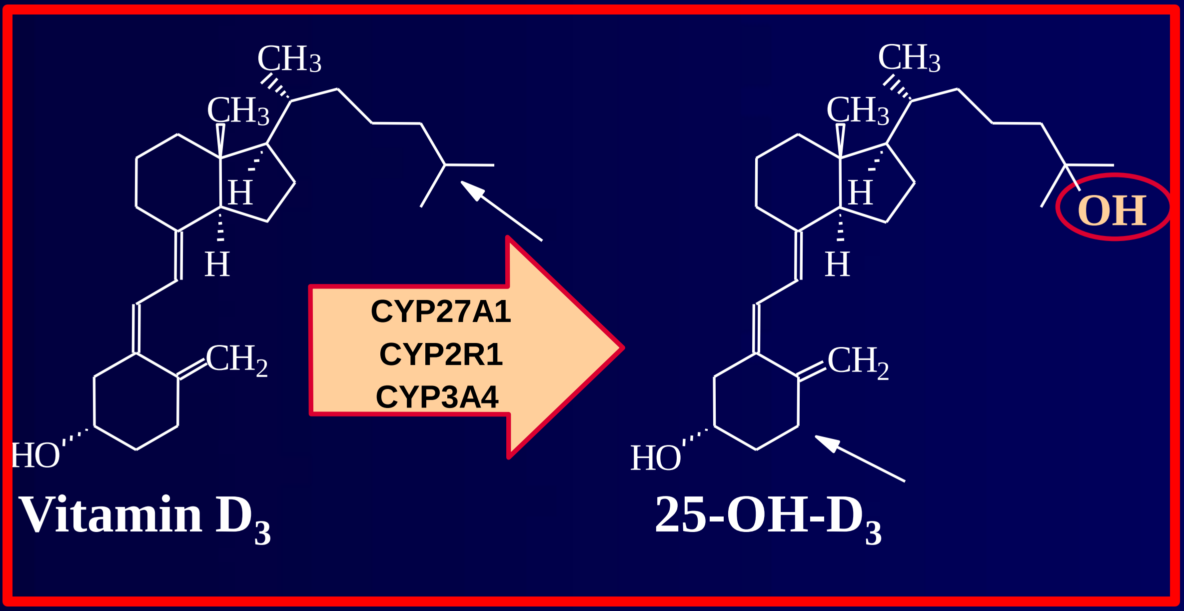
25-OH-D_3

1α,25-$(OH)_2D_3$

Calcitriol

Similar pathway exists for vitamin D_2

Metabolism of Vitamin D_3



Perfused Liver reveals two 25-Hydroxylases

Table 1. Two modes of the 25-hydroxylation reaction of vitamin D_3 in rat liver.

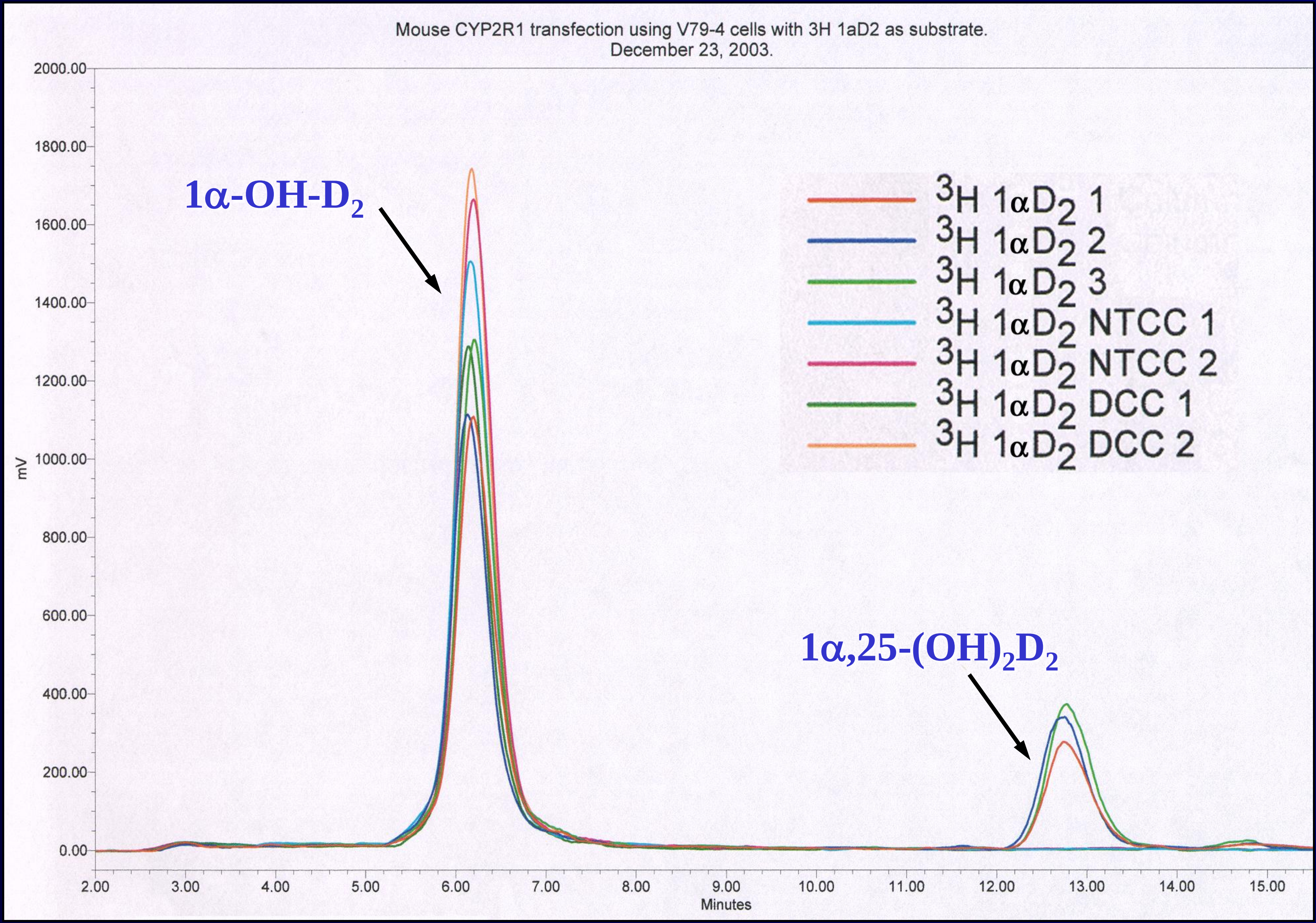
Mode of Reaction	I	II
Apparent Km	5.6×10^{-8} M	5.0×10^{-6} M
Substrate specificity	Specific	Non-specific
Amounts of Enzyme	limited	large
(Binding capacity)	< 1.3 nmoles (20 IU／200 gm)	> 1.3 nmoles
Velocity	large	small

Suda et al. (1977) Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism. 201-213

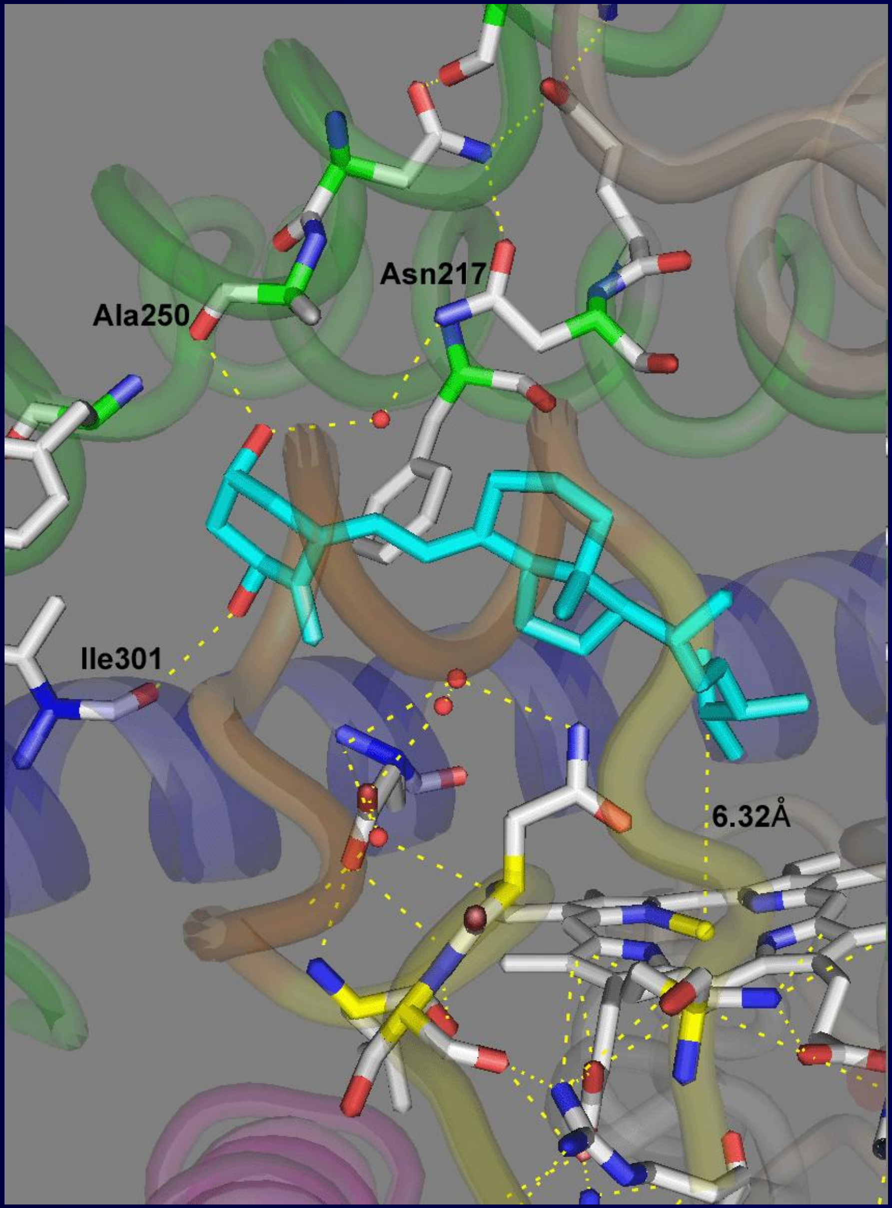
CYP2R1

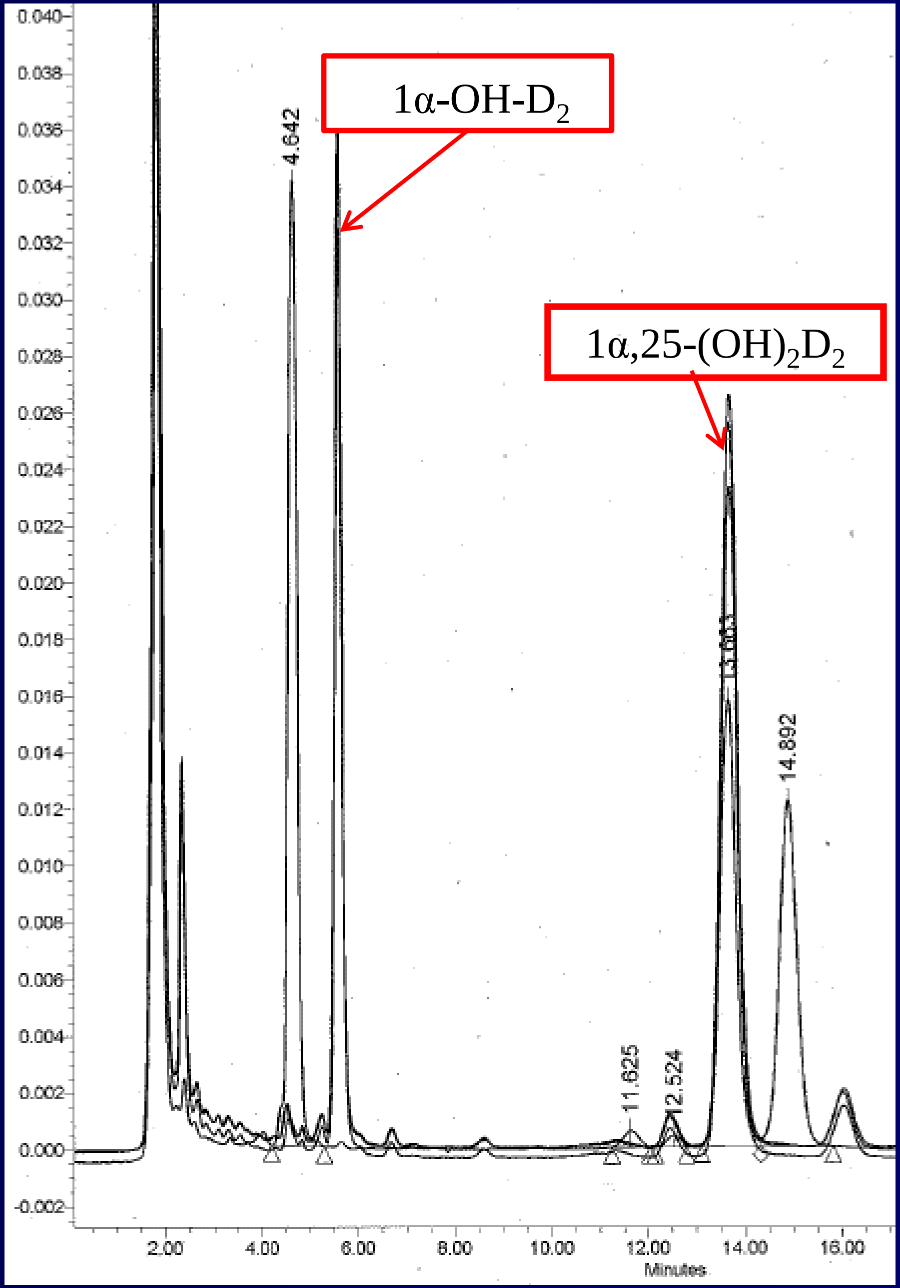
- LIVER MICROSOMAL VITAMIN D 25-HYDROXYLASE
- SUBSTRATES INCLUDE VITAMINS D_3 & D_2 & ANTICANCER PRODRUGS (eg 1α-OH-D_2)
- MUTATION IN hCYP2R1 AT L99P CAUSES RICKETS
- WORKS AT NANOMOLAR SUBSTRATE & PROBABLY THE PHYSIOLOGICALLY RELEVANT VIT.D-25-HYDROXYLASE
- SGC (TORONTO) CRYSTALLISED A FUNCTIONAL HUMAN CYP2R1 WITH VITAMIN D_3 IN ACTIVE SITE

25-Hydroxylation of 1α-OH-D_2 by Mouse cyp2R1



1α-OH-D_2 in CYP2R1





From : Strushkevich N, Usanov SA, Plotnikov AN, Jones G, Park H-W (2008) Structural Analysis of CYP2R1 in complex with vitamin D_3. J Mol Biol **380**: 95-106

Perfused Liver reveals two 25-Hydroxylases

Table 1. Two modes of the 25-hydroxylation reaction of vitamin D_3 in rat liver.

Mode of Reaction	CYP2R1	CYP27A1 CYP3A4
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Substrate specificity	Specific	Non-specific
Amounts of Enzyme	limited	large
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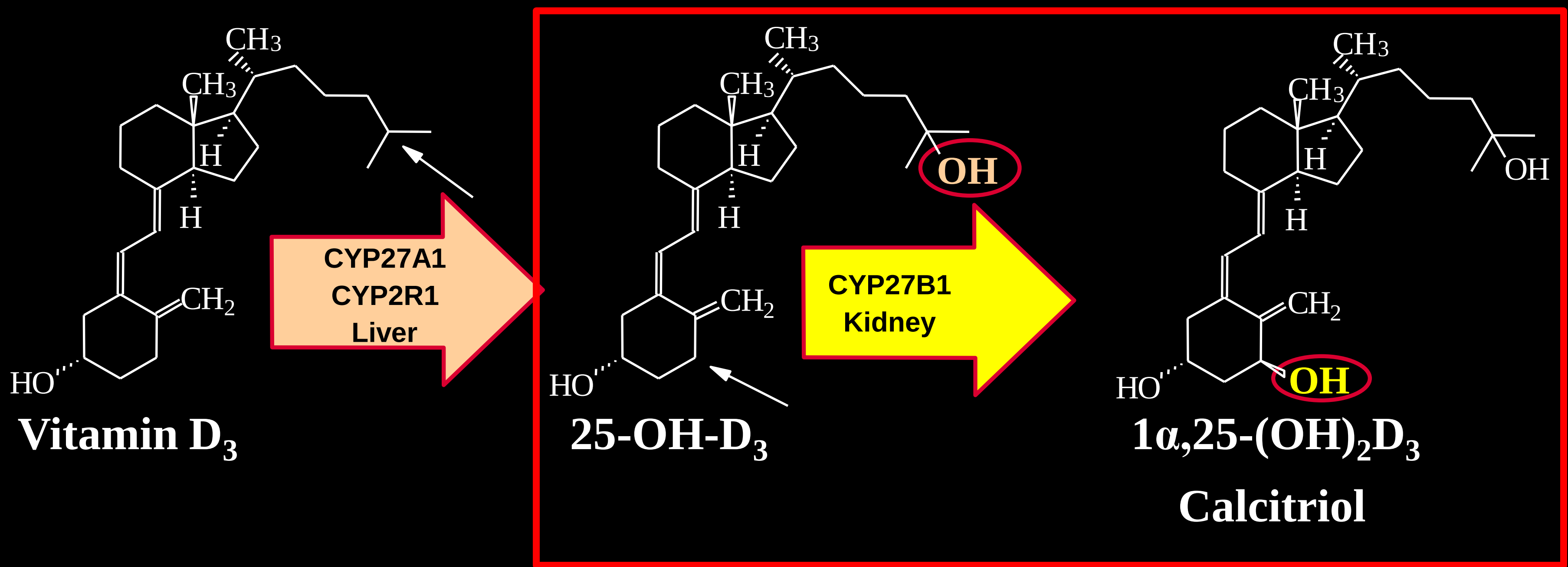
Suda et al. (1977) Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism. 201-213

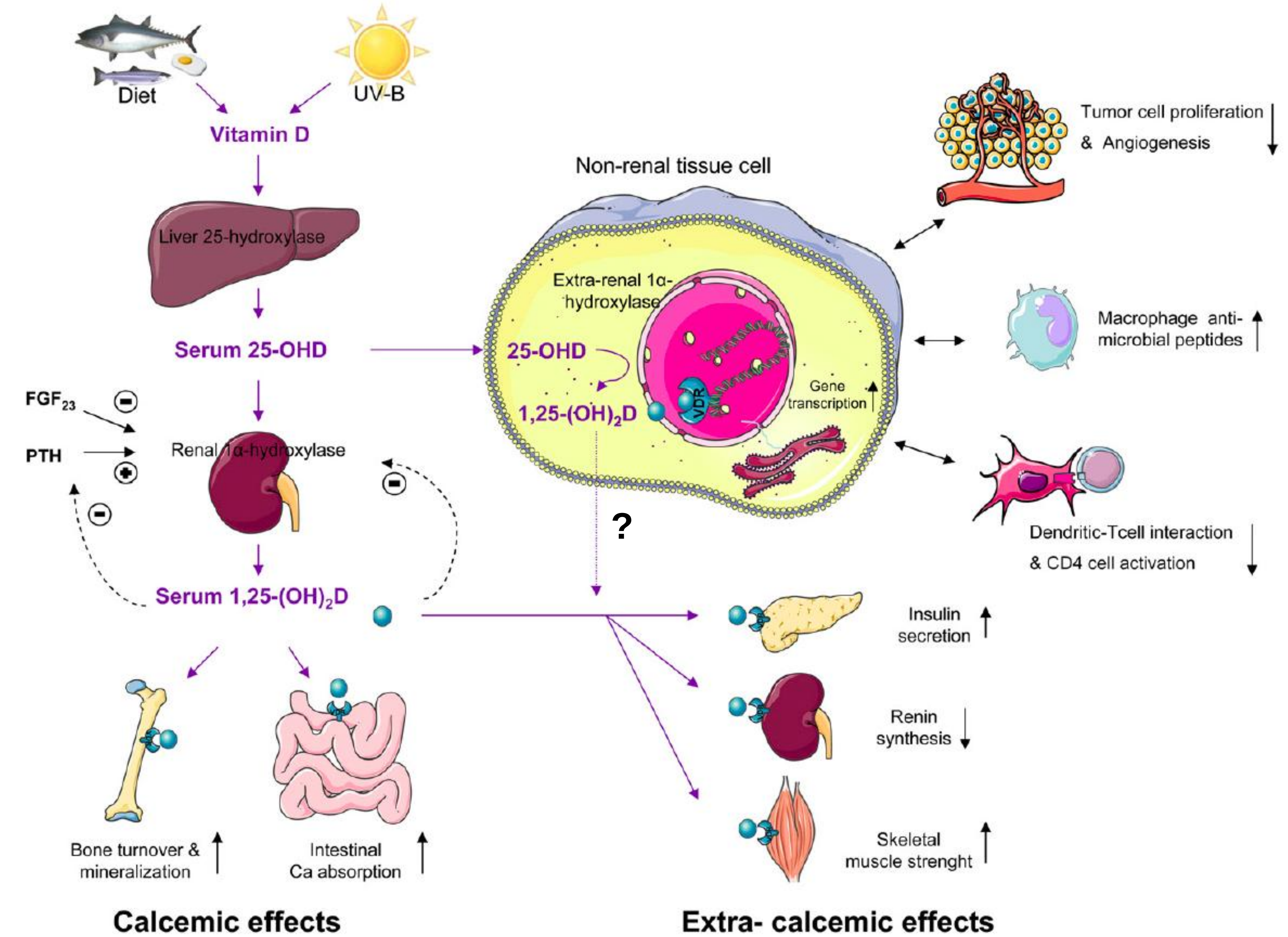
Metabolism of Vitamin D_3



Similar pathway exists for vitamin D_2

CYP27B1

- KIDNEY MITOCHONDRIAL 1α-HYDROXYLASE
- SUBSTRATES INCLUDE 25-OH-D_3 & 25-OH-D_2
- MUTATIONS IN hCYP27B1 CAUSE VDDR-RICKETS TYPE 1
- PROBES & ANTIBODIES REVEAL PRESENCE OF CYP27B1 IN EXTRA-RENAL TISSUES eg SKIN, MONOCYTE
- REGULATION DIFFERENT IN DIFFERENT TISSUES:
  A) KIDNEY CYP27B1–PTH & FGF-23; Ca & PO_4
  B) EXTRA-RENAL CYP27B1 -- CYTOKINES



Janssens W et al (2009) Am J Respir Crit Care Med 179:630-6.

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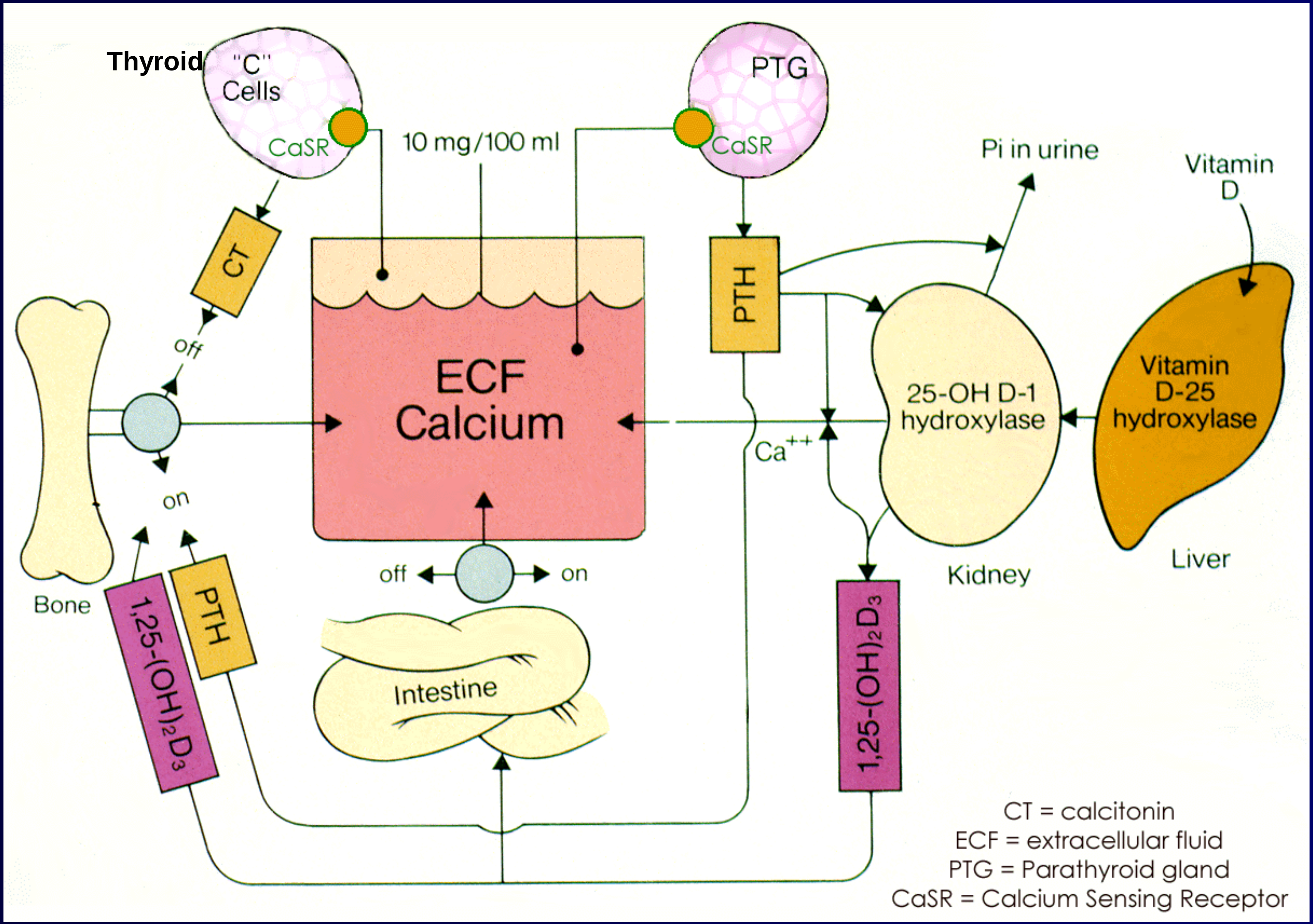
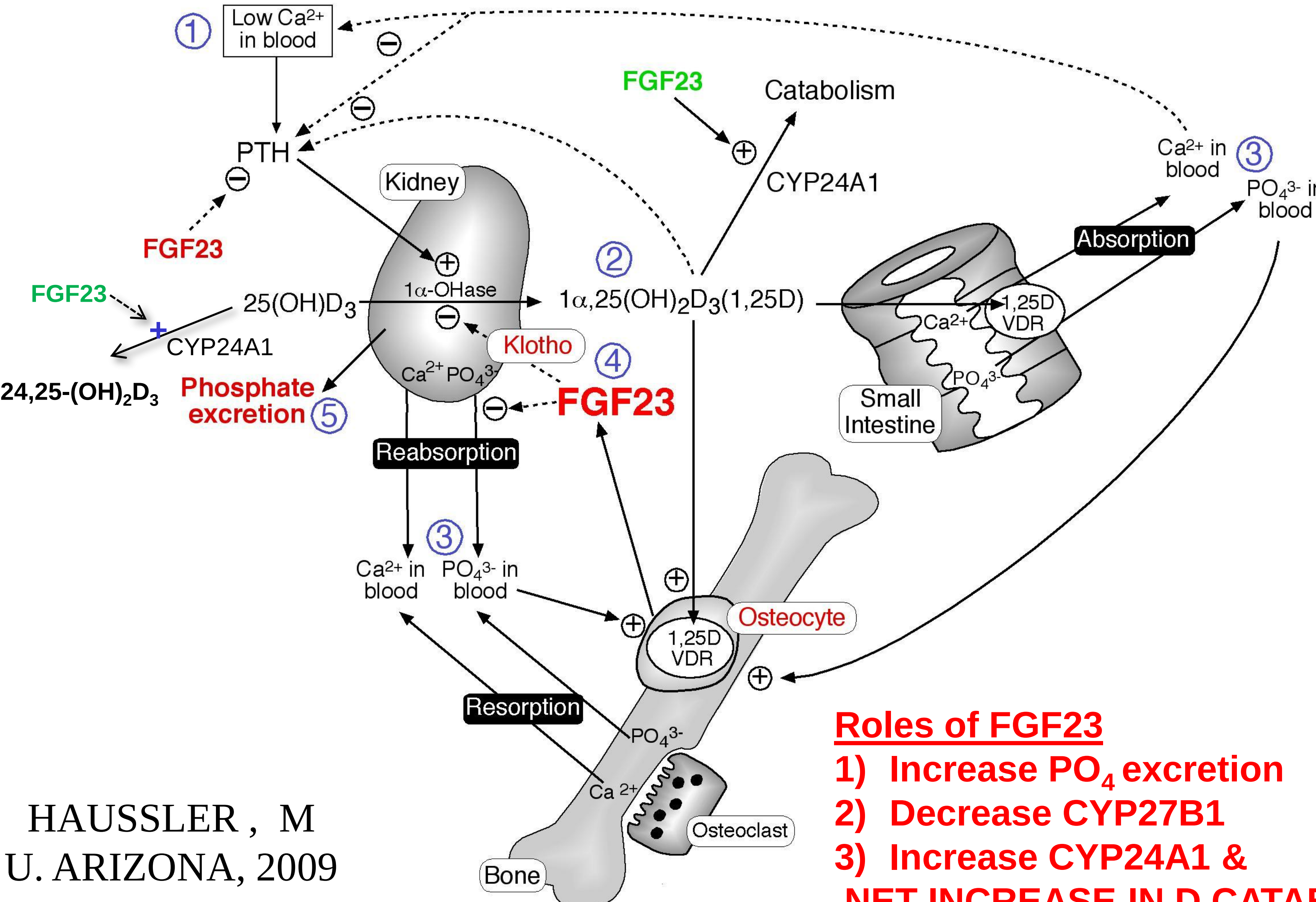
Plasma Calcium Homeostasis



CENTRAL ROLE OF FGF23 IN PHOSPHATE & VITAMIN D HOMEOSTASIS



Roles of FGF23

1) Increase PO_4 excretion
2) Decrease CYP27B1
3) Increase CYP24A1 & NET INCREASE IN D CATABOLISM

HAUSSLER , M
U. ARIZONA, 2009

CYP24A1

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

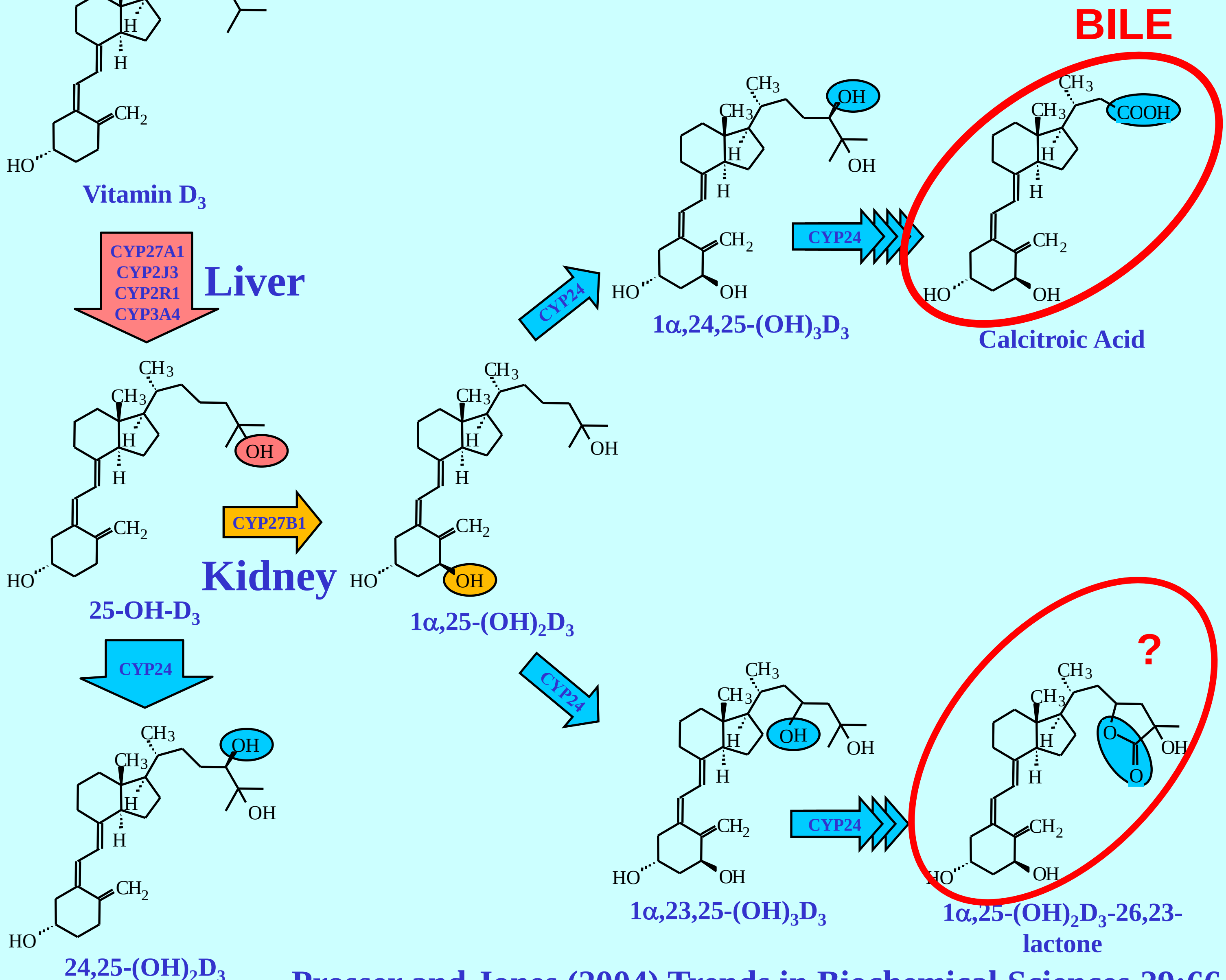
- SUBSTRATES INCLUDE 25-OH-D_3 & 1α,25-$(OH)_2D_3$
  & CYP24 IS A MULTICATALYTIC ENZYME

- INDUCED BY 1α,25-$(OH)_2D_3$ & FGF-23 IN TARGET CELLS

- CYP24A1 KNOCKOUT MOUSE SHOWS 50% LETHALITY

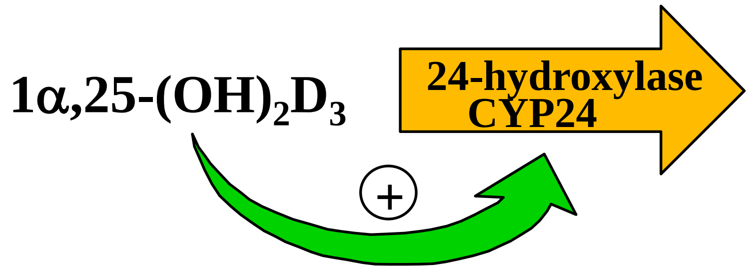
- MAJOR ROLE IS CATABOLIC AND EXISTS TO ATTENUATE ACTION OF CALCITRIOL INSIDE TARGET CELLS

Metabolism of Vitamin D



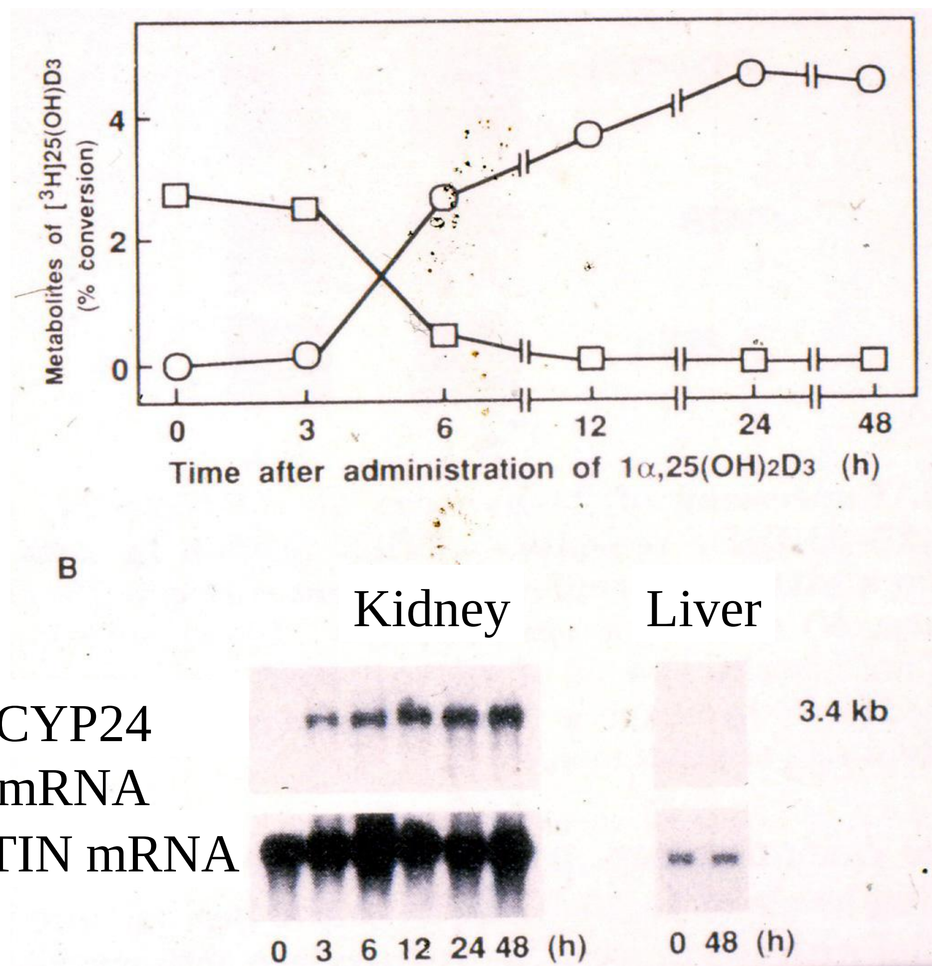
Prosser and Jones (2004) Trends in Biochemical Sciences 29:664-73.

CYP24 IS INDUCED BY ITS SUBSTRATE



Transcriptional up-regulation

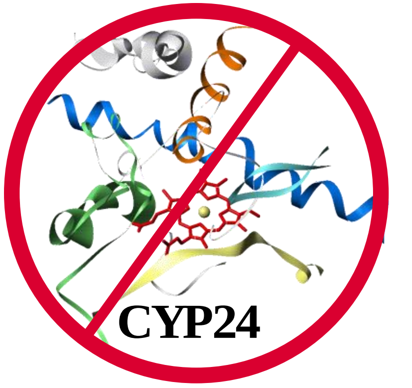
-

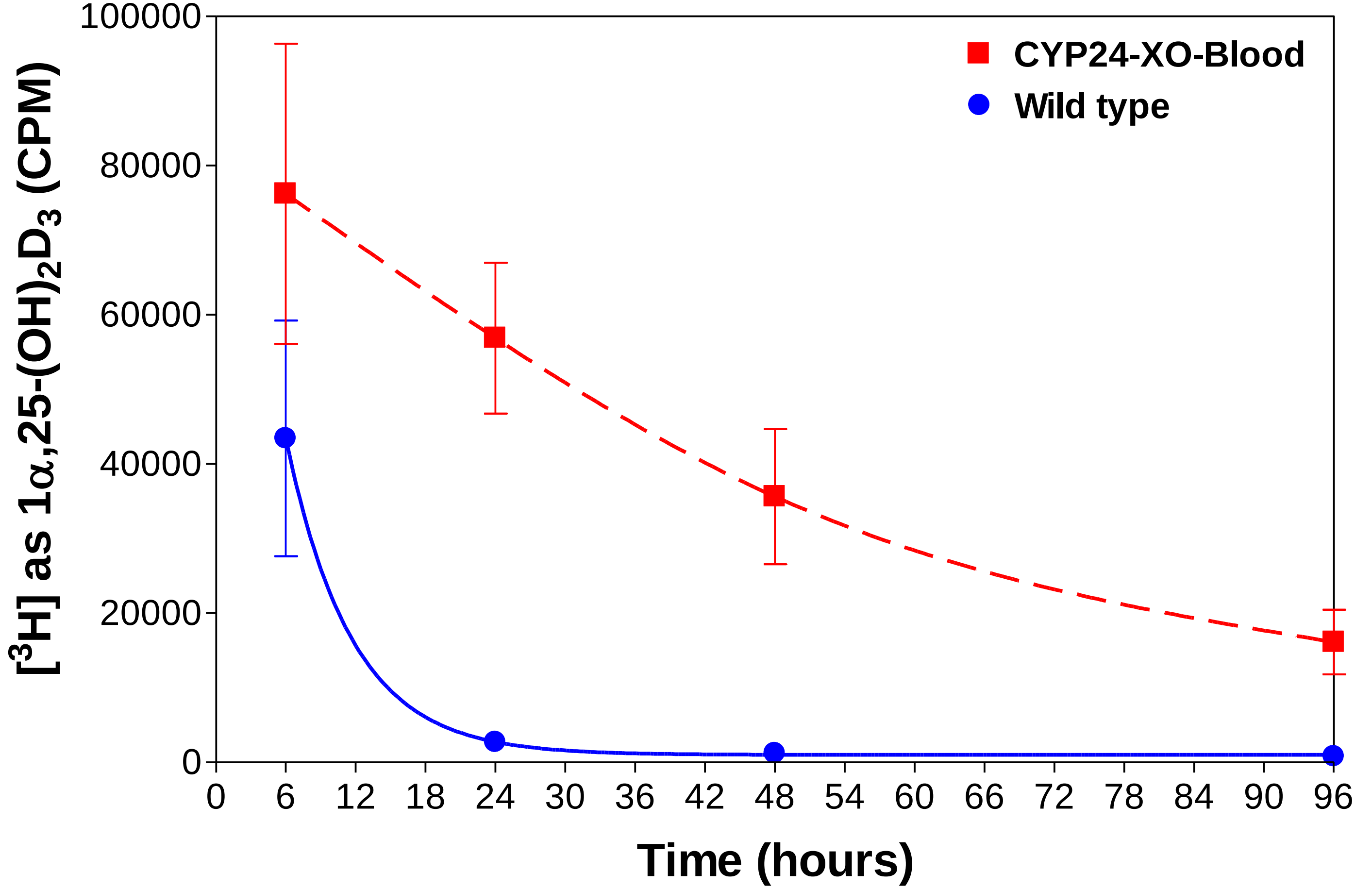


CYP24 mRNA

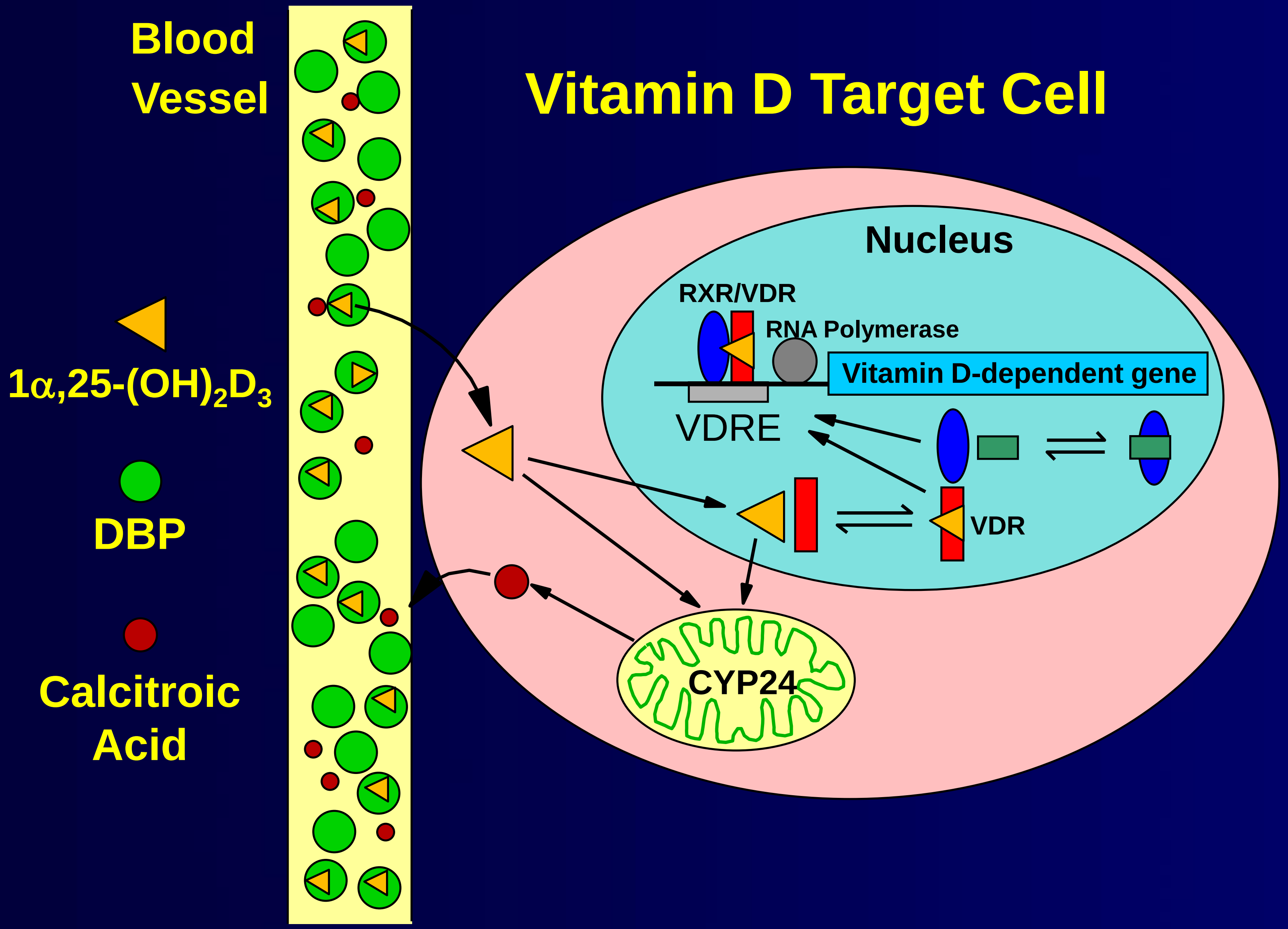
β-ACTIN mRNA

$[^3H]1\alpha,25\text{-}(OH)_2D_3$ in Blood of Wild type and Cyp24-XO Mice



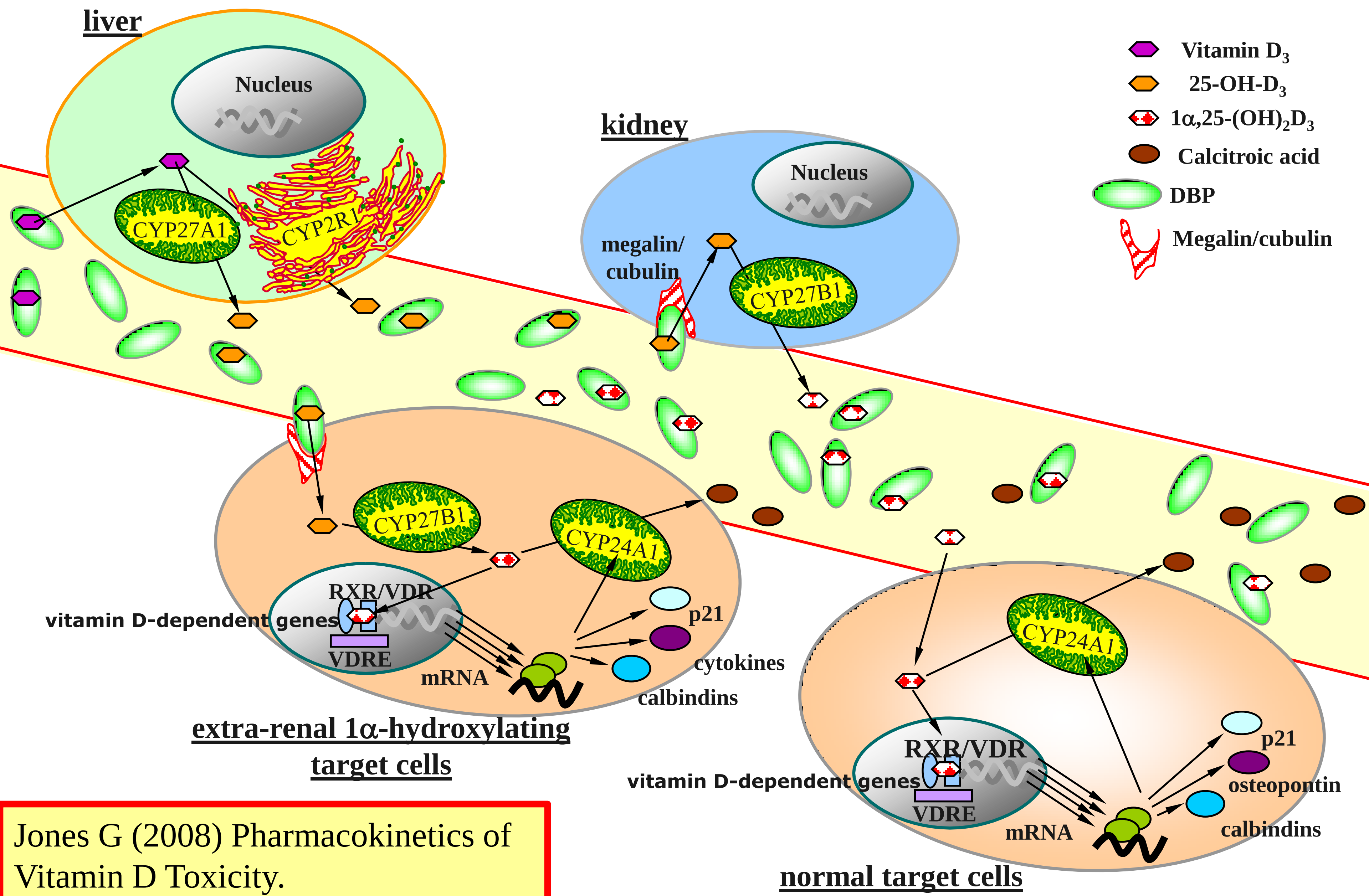


Masuda et al Endocrinology, 2005



Blood Vessel
Vitamin D Target Cell
Nucleus
RXR/VDR
RNA Polymerase
Vitamin D-dependent gene
VDRE
VDR
CYP24
1α,25-(OH)$_2$D$_3$
DBP
Calcitroic Acid

Vitamin D –Endocrine & Intracrine System



Jones G (2008) Pharmacokinetics of Vitamin D Toxicity. Amer J Clin Nutr 88: 582S-586S.

Vitamin D Metabolism & Function

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- **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**
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  - Vitamin D Deficiency may underlie several major diseases
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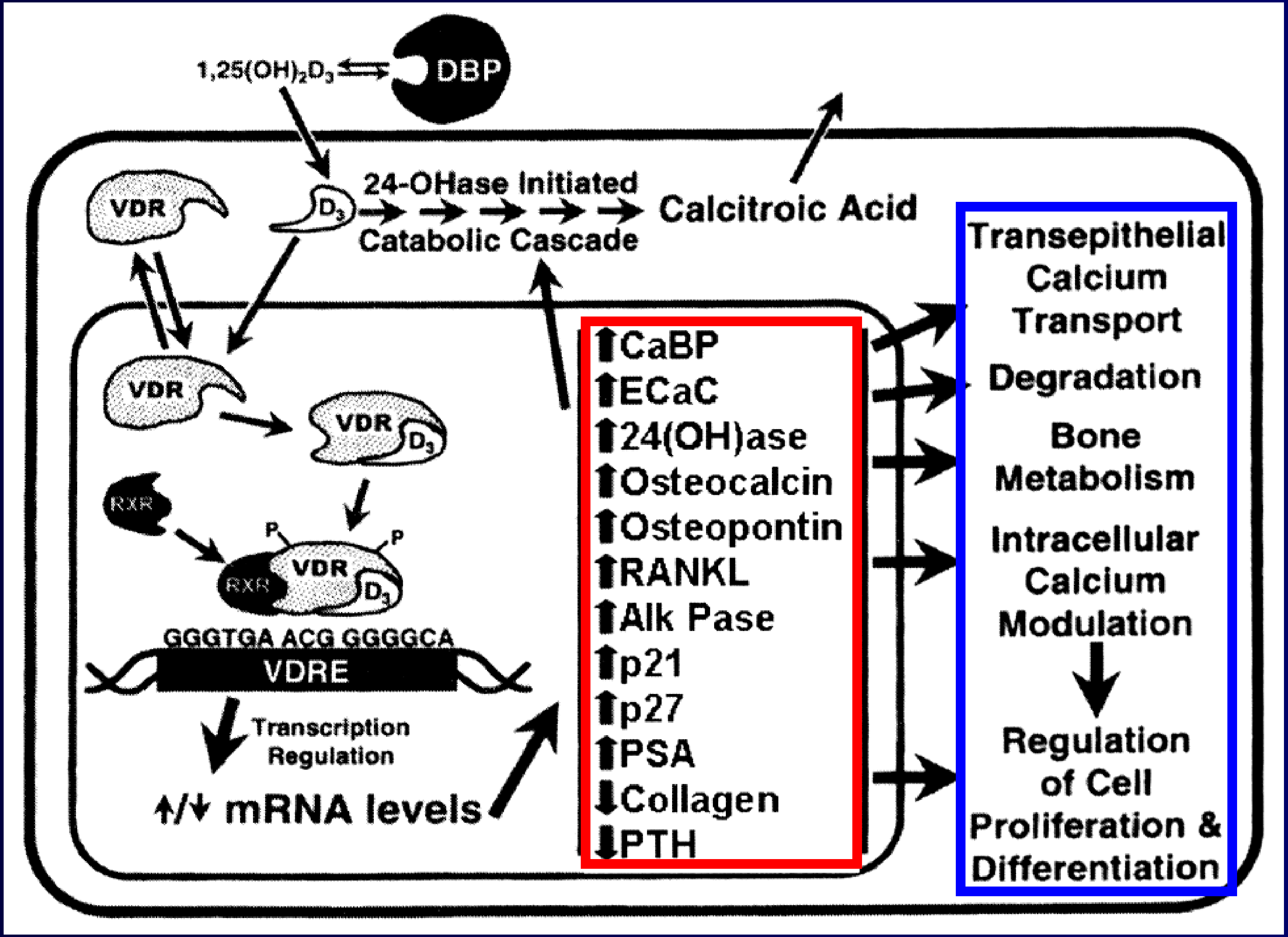
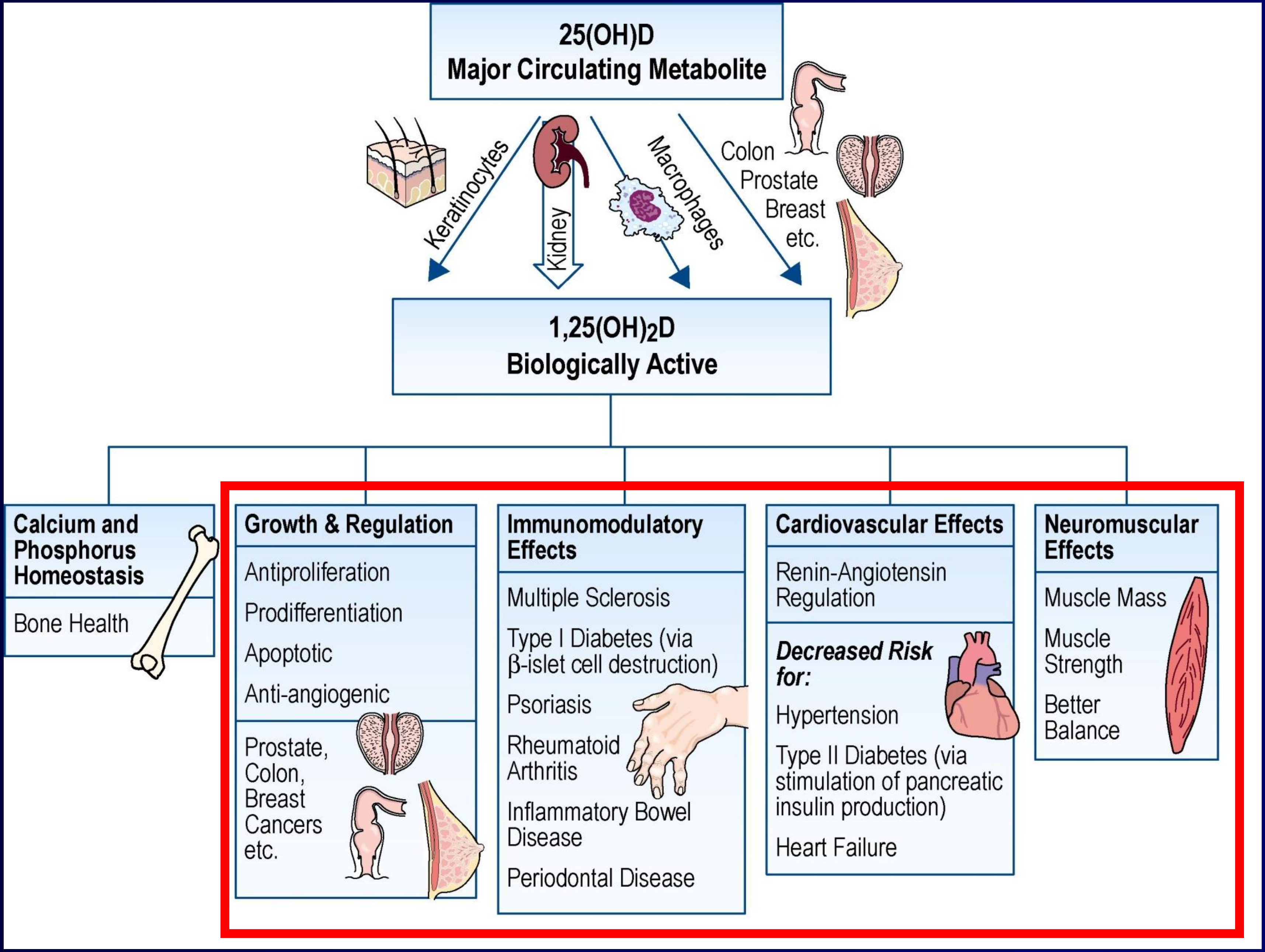
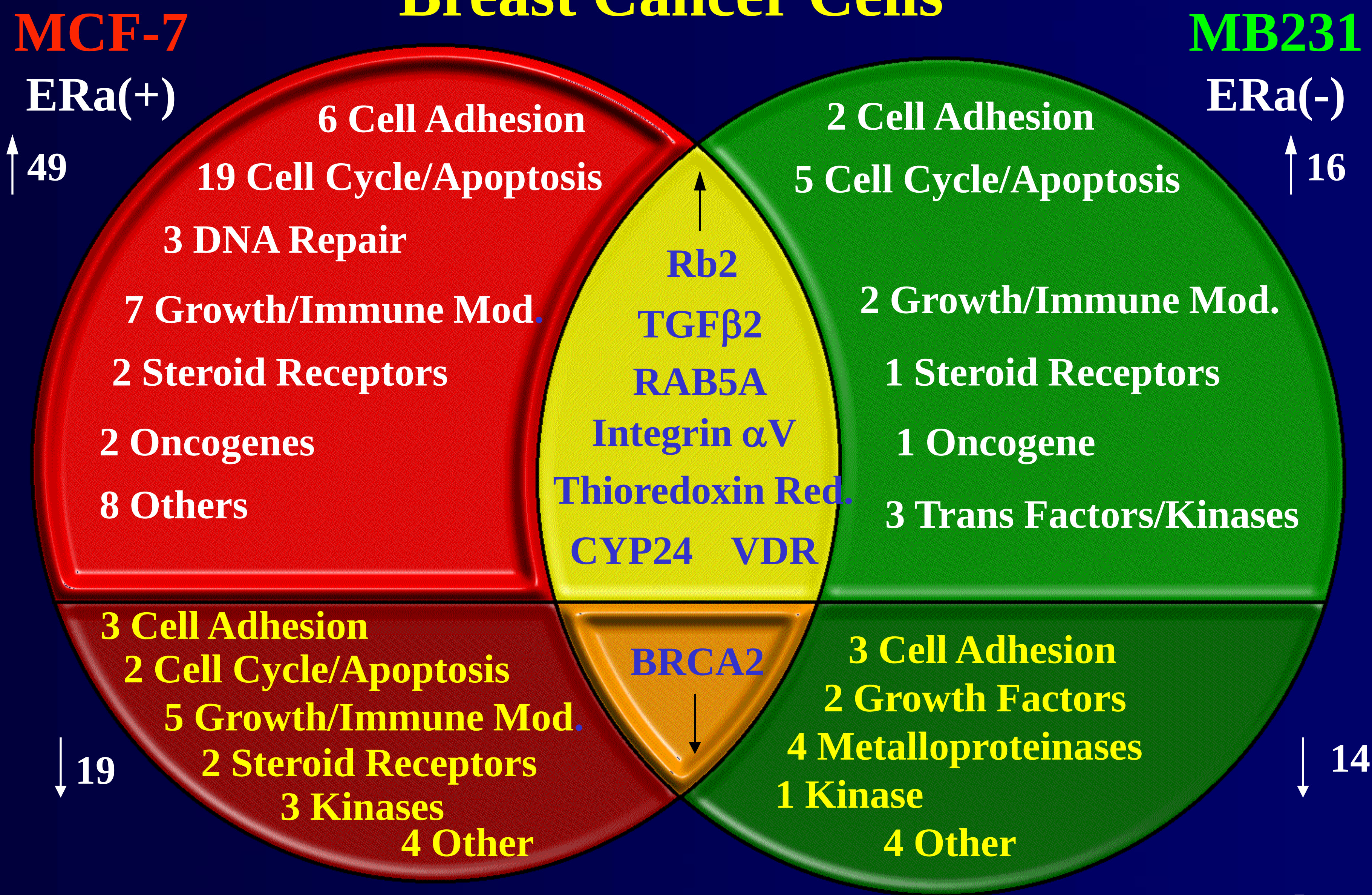
Mechanism of Action of Vitamin D





Holick M (2006) Mayo Clin Proc 81:353-73.

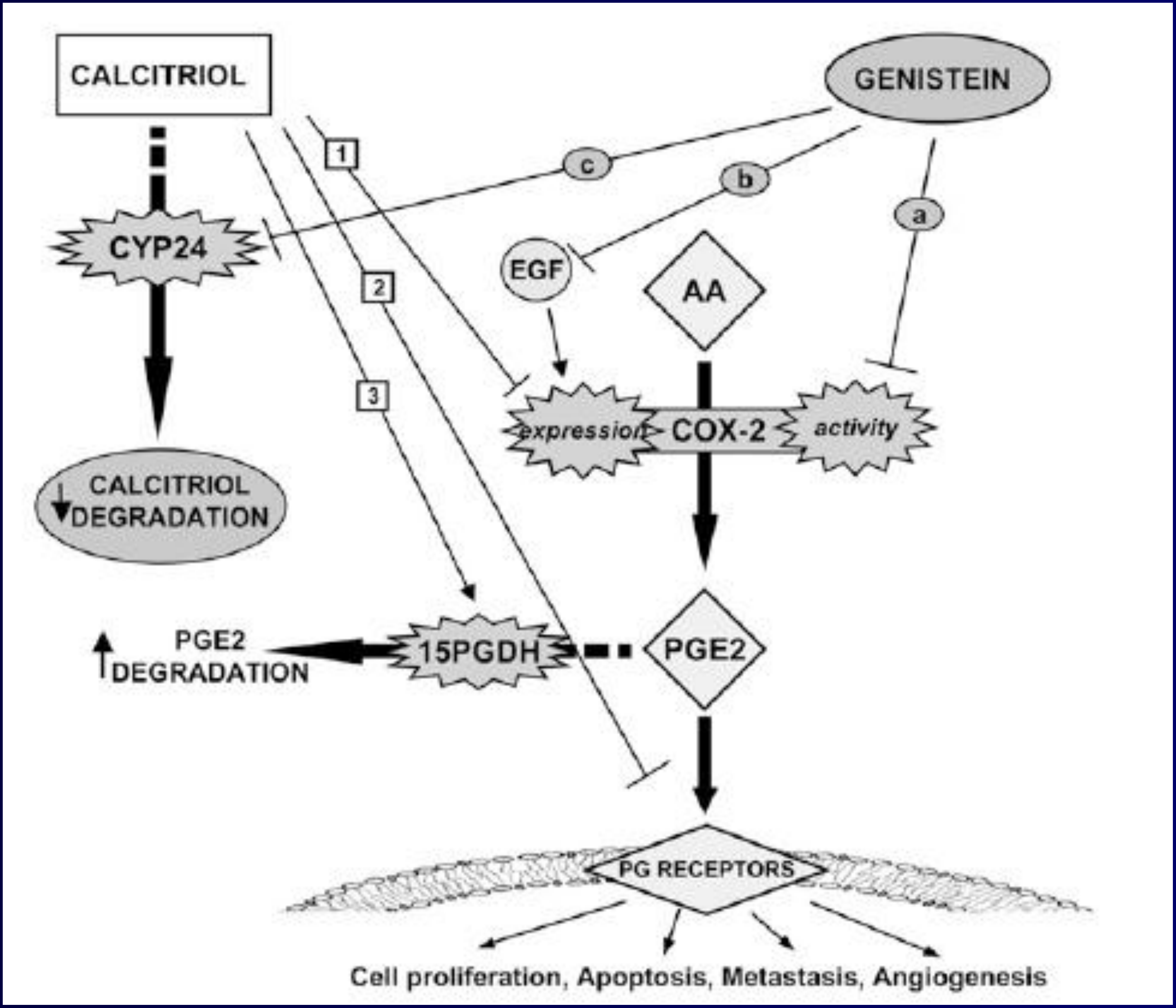
Microarray Analysis of Calcitriol Treatment Breast Cancer Cells



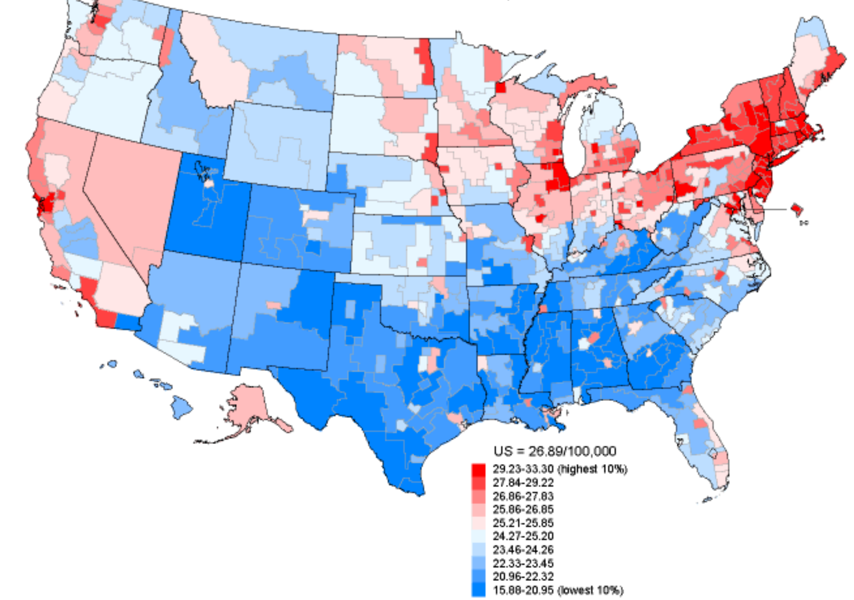
Swami et al., (2003) Breast Cancer Res. Treat. 80:49-62.

2000 Gene Probes

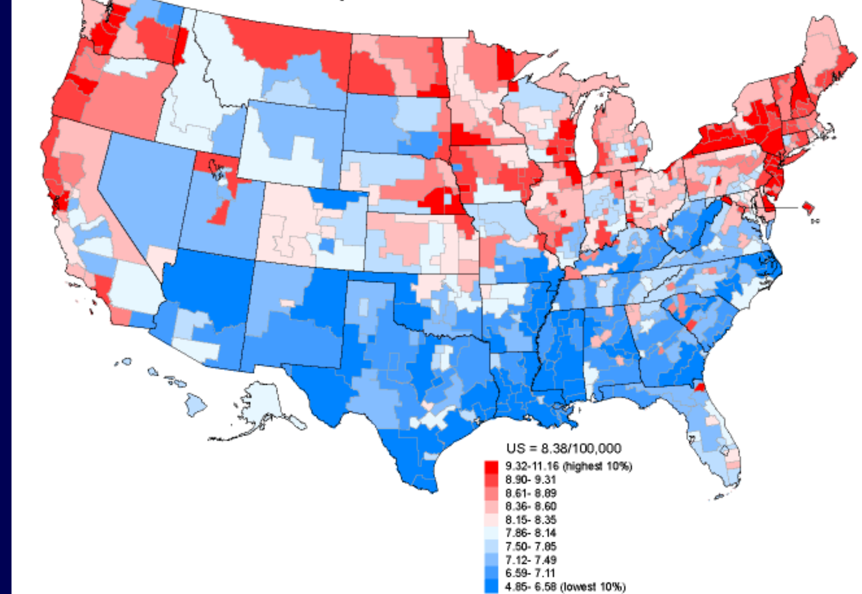
Calcitriol blocks prostaglandin pathways



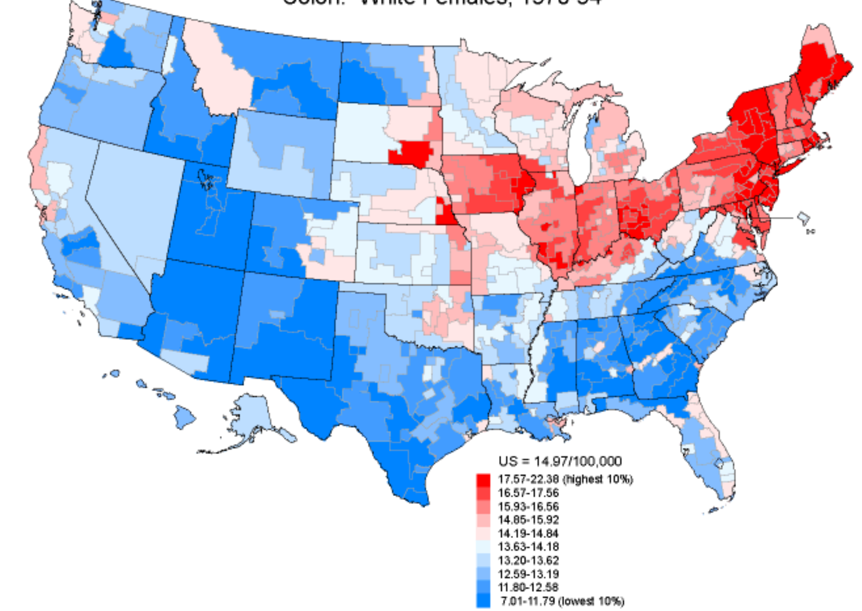
Cancer Mortality Rates by State Economic Area (Age-adjusted 1970 US Population)
Breast: White Females, 1970-94



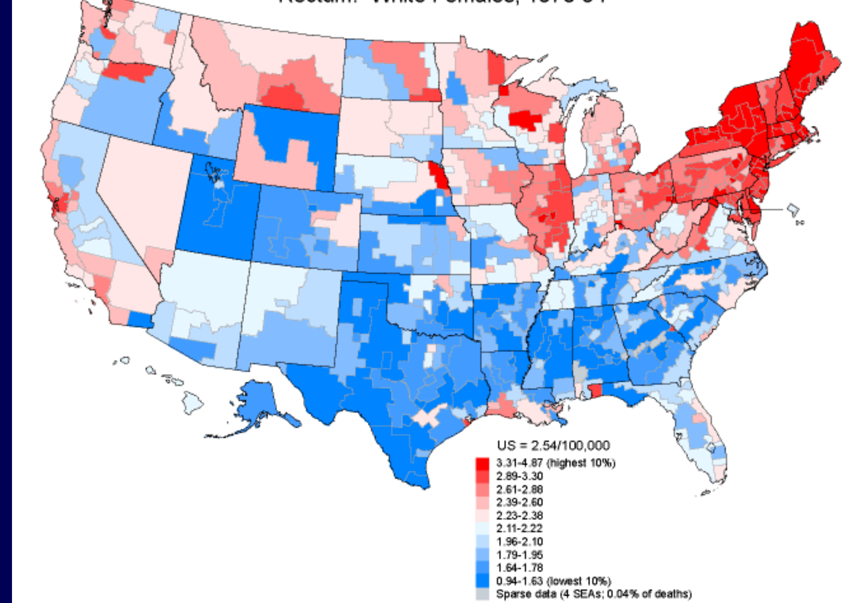
Cancer Mortality Rates by State Economic Area (Age-adjusted 1970 US Population)
Ovary: White Females, 1970-94

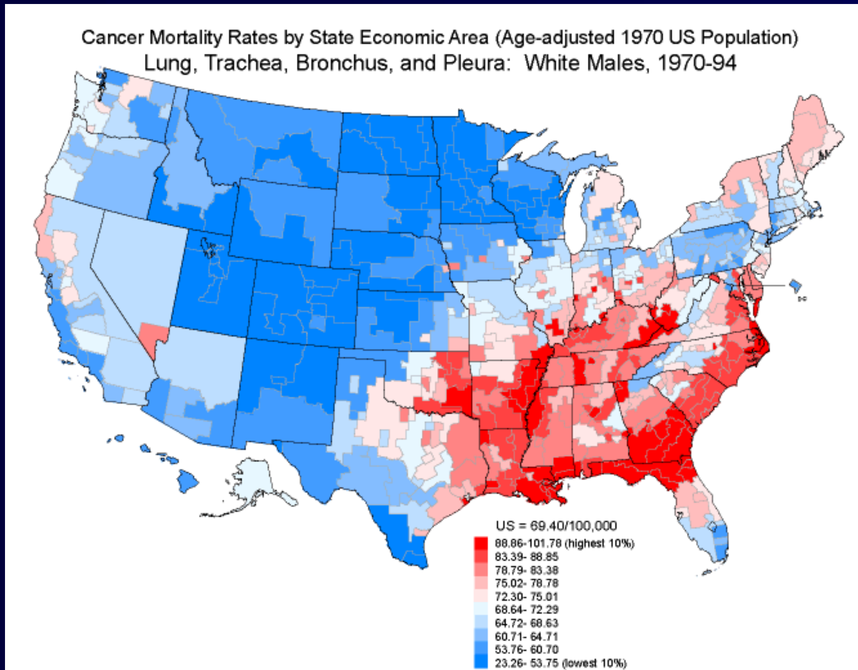


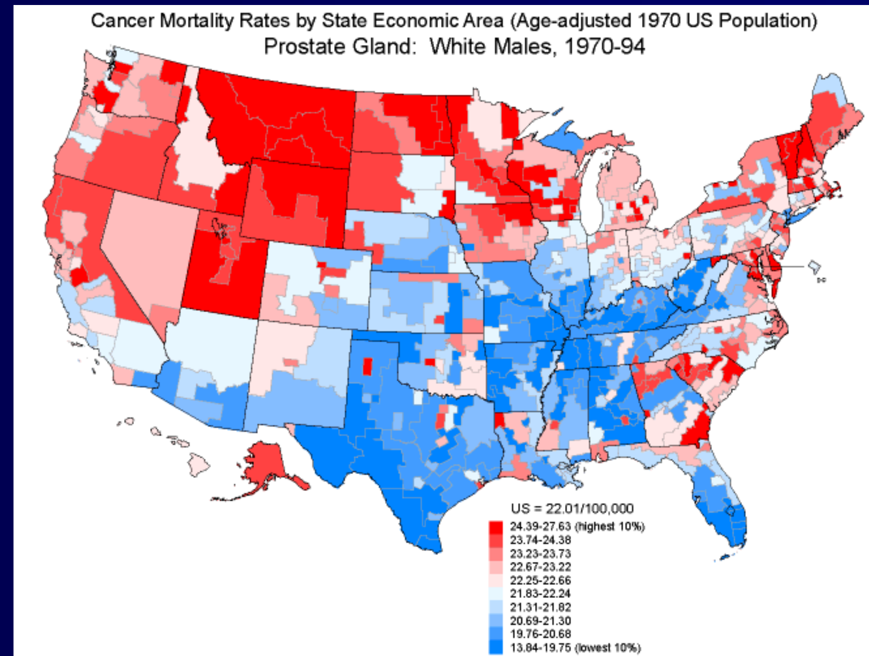
Cancer Mortality Rates by State Economic Area (Age-adjusted 1970 US Population)
Colon: White Females, 1970-94

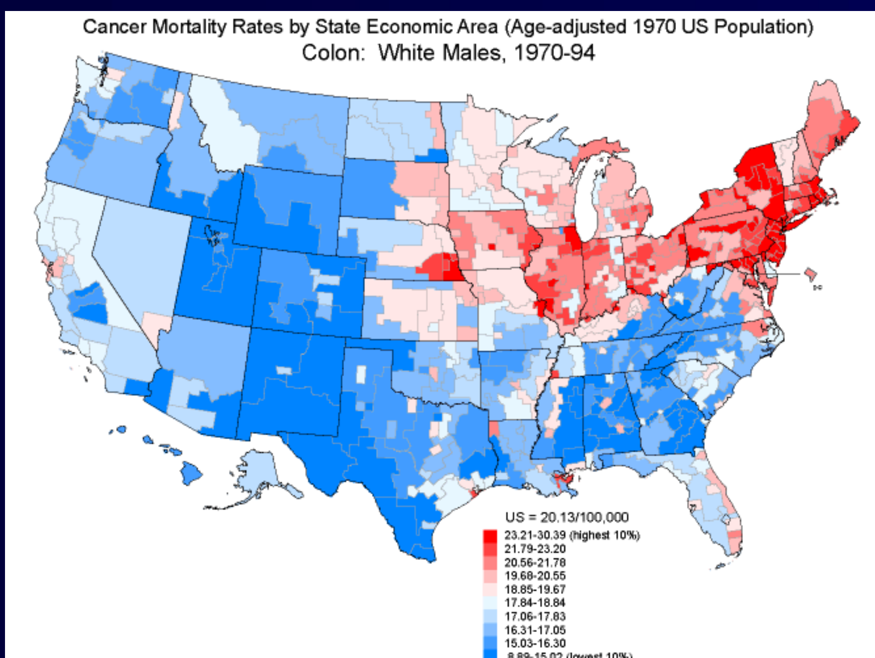


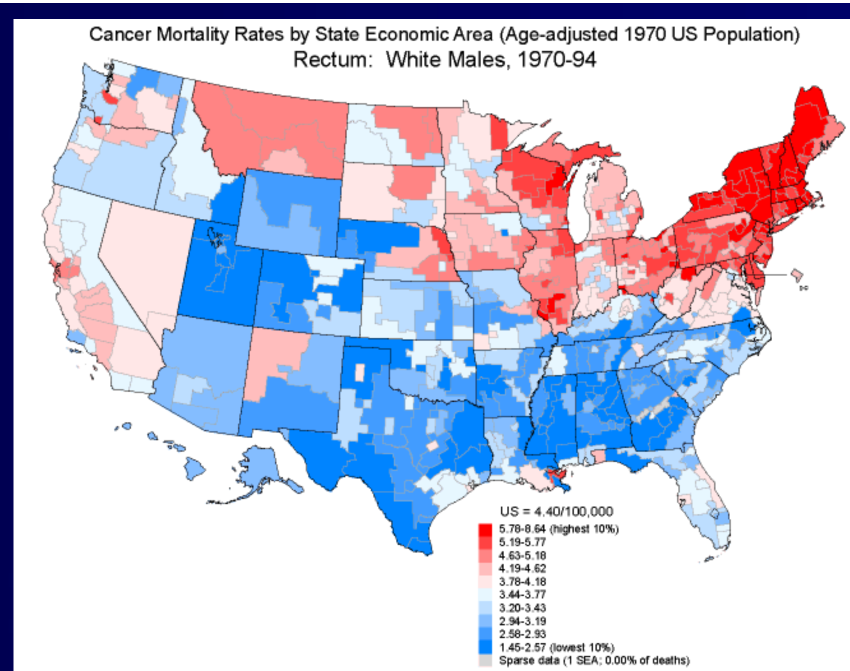
Cancer Mortality Rates by State Economic Area (Age-adjusted 1970 US Population)
Rectum: White Females, 1970-94



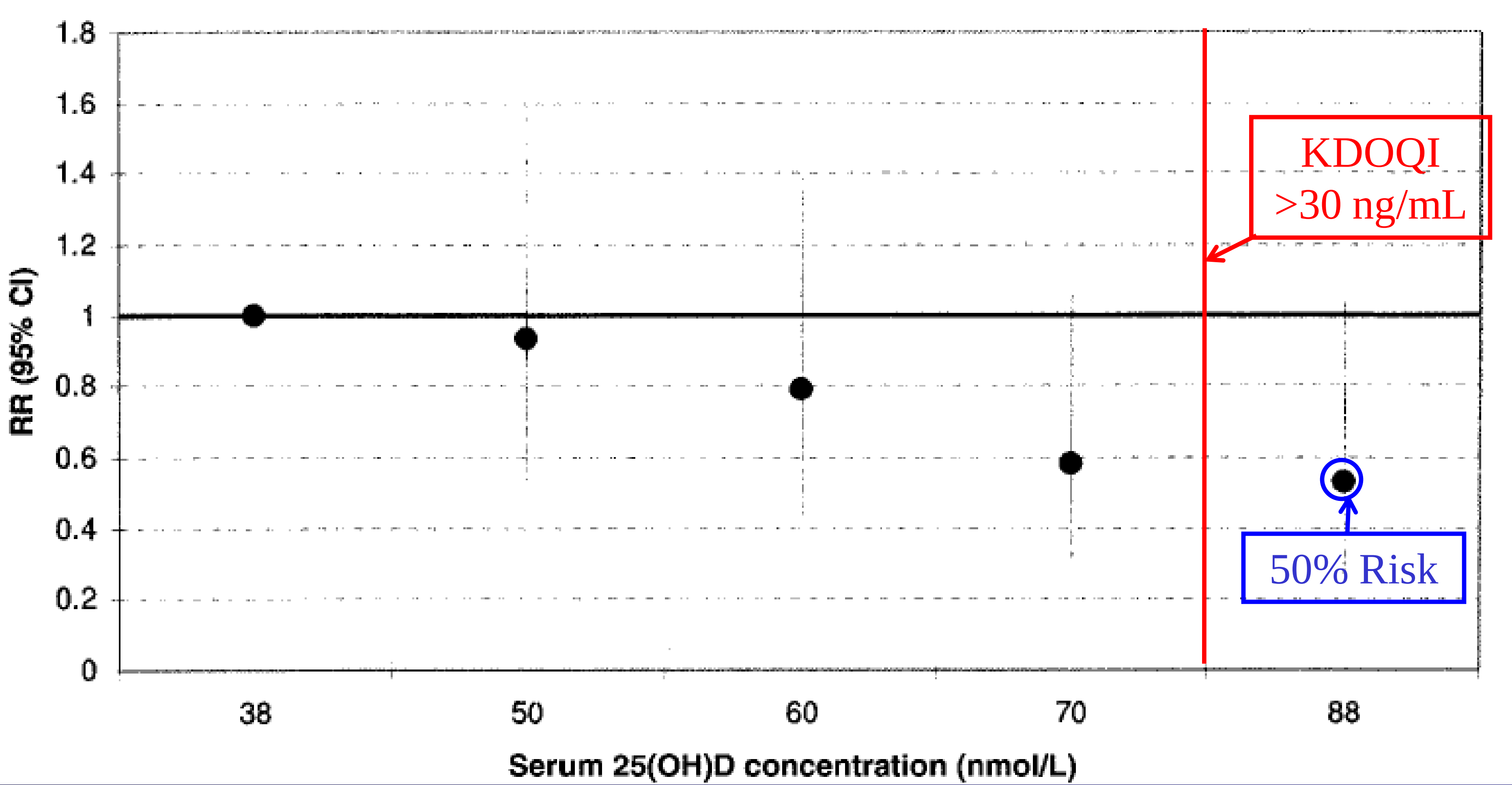

Cancer Mortality Rates by State Economic Area (Age-adjusted 1970 US Population)
Lung, Trachea, Bronchus, and Pleura: White Males, 1970-94
US = 69.40/100,000
88.86-101.78 (highest 10%)
83.39- 88.85
78.79- 83.38
75.02- 78.78
72.30- 75.01
68.64- 72.29
64.72- 68.63
60.71- 64.71
53.76- 60.70
23.26- 53.75 (lowest 10%)


Cancer Mortality Rates by State Economic Area (Age-adjusted 1970 US Population)
Prostate Gland: White Males, 1970-94
US = 22.01/100,000
24.39-27.63 (highest 10%)
23.74-24.38
23.23-23.73
22.67-23.22
22.25-22.66
21.83-22.24
21.31-21.82
20.69-21.30
19.76-20.68
13.84-19.75 (lowest 10%)


Cancer Mortality Rates by State Economic Area (Age-adjusted 1970 US Population)
Colon: White Males, 1970-94
US = 20.13/100,000
23.21-30.39 (highest 10%)
21.79-23.20
20.56-21.78
19.68-20.55
18.85-19.67
17.84-18.84
17.06-17.83
16.31-17.05
15.03-16.30
8.89-15.02 (lowest 10%)


Cancer Mortality Rates by State Economic Area (Age-adjusted 1970 US Population)
Rectum: White Males, 1970-94
US = 4.40/100,000
5.78-8.64 (highest 10%)
5.19-5.77
4.63-5.18
4.19-4.62
3.78-4.18
3.44-3.77
3.20-3.43
2.94-3.19
2.58-2.93
1.45-2.57 (lowest 10%)
Sparse data (1 SEA; 0.00% of deaths)

Relative risk of Colon Cancer vs Serum 25-OH-D



Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes[1–3] *Am J Clin Nutr* 2006;84:18–28.

Heike A Bischoff-Ferrari, Edward Giovannucci, Walter C Willett, Thomas Dietrich, and Bess Dawson-Hughes

Am J Clin Nutr 2007; 85:1586-91

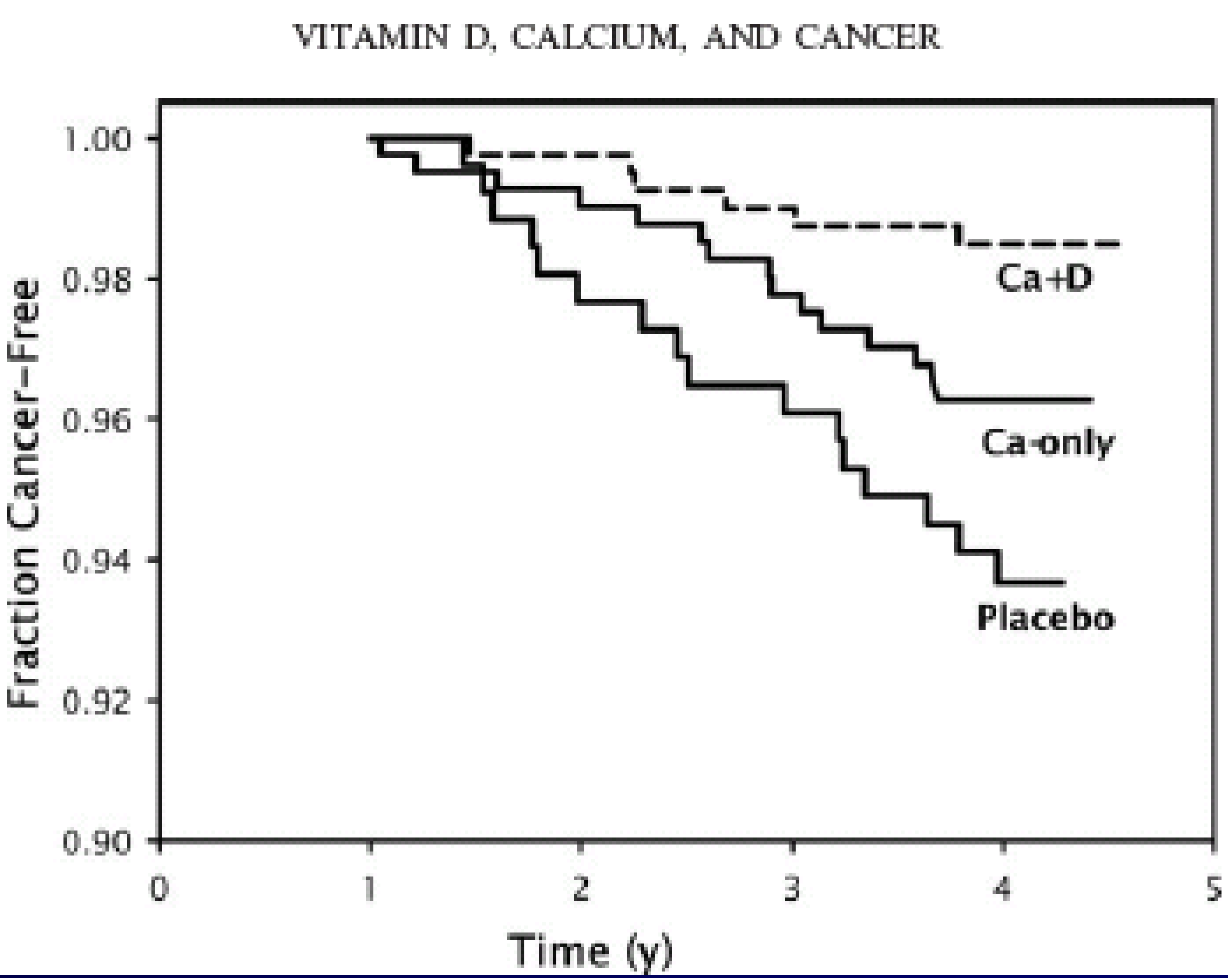
Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial[1,2]

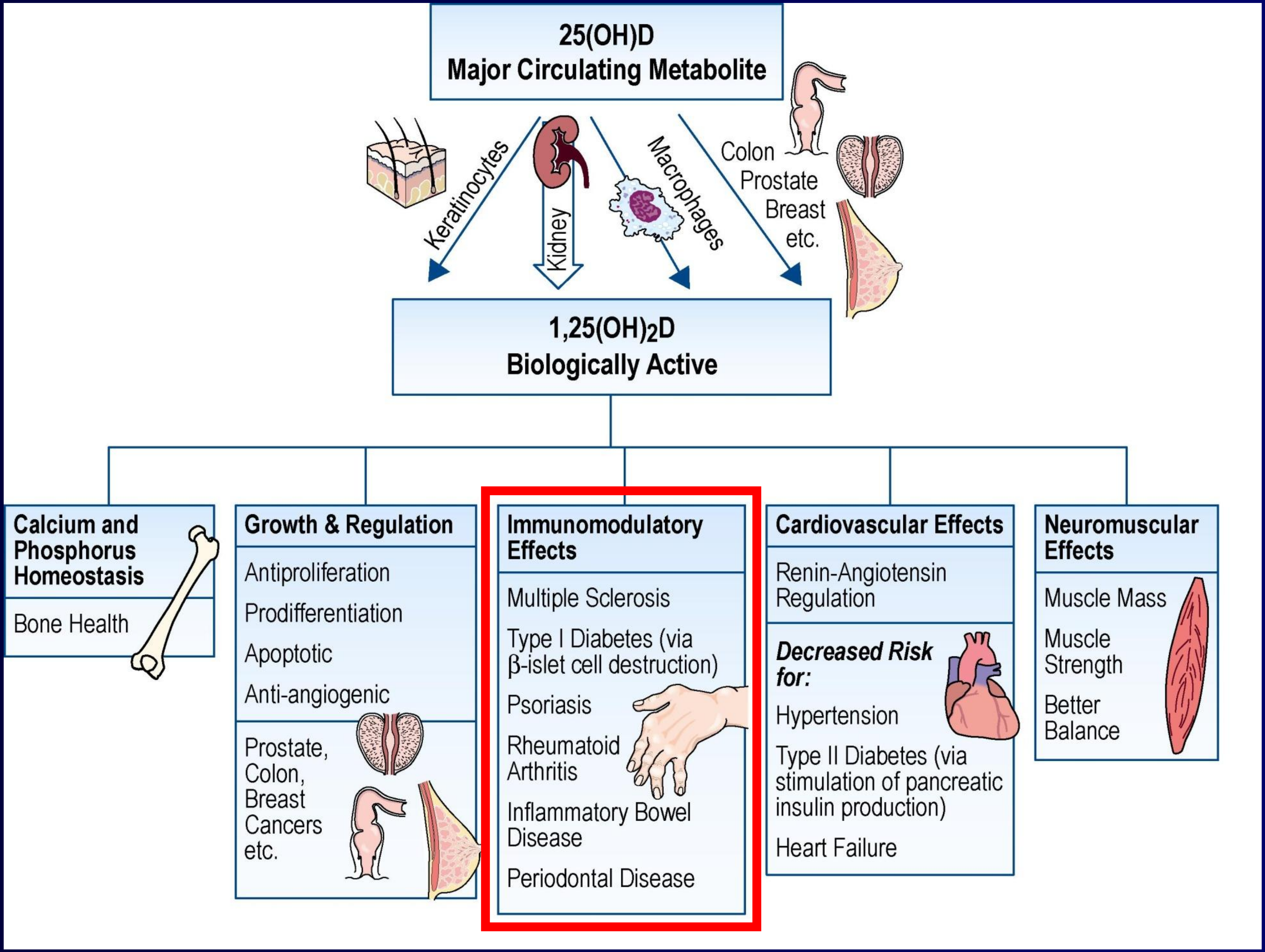
Joan M Lappe, Dianne Travers-Gustafson, K Michael Davies, Robert R Recker, and Robert P Heaney

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

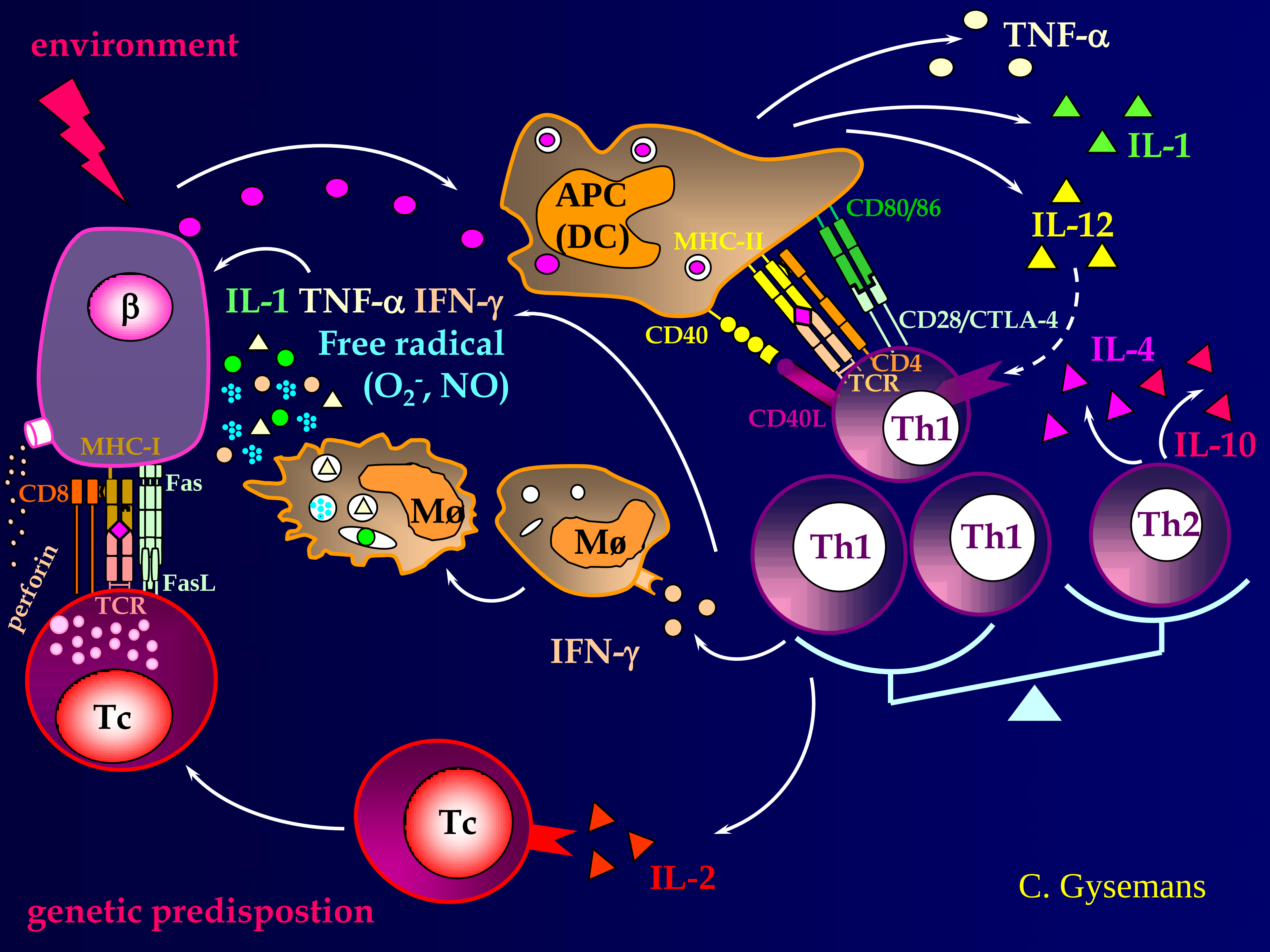
BLOOD 25-OH-D level

Ca+D = 72 →96 nmol/L
Ca ONLY = 71 nmol/L
PLACEBO = 71 nmol/L

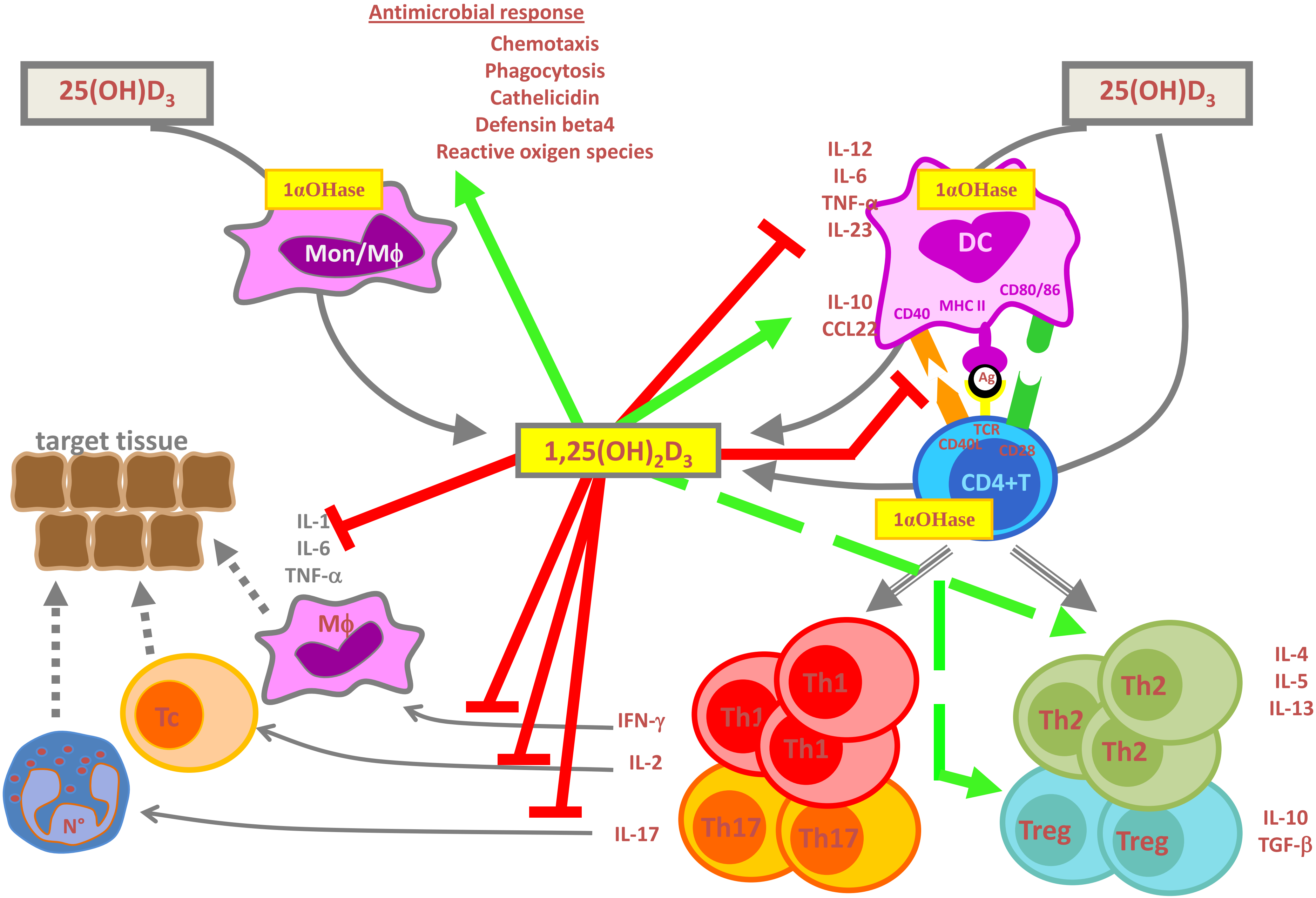




25(OH)D
Major Circulating Metabolite
Keratinocytes
Kidney
Macrophages
Colon
Prostate
Breast
etc.
1,25(OH)2D
Biologically Active
Calcium and Phosphorus Homeostasis
Bone Health
Growth & Regulation
Antiproliferation
Prodifferentiation
Apoptotic
Anti-angiogenic
Prostate, Colon, Breast Cancers etc.
Immunomodulatory 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



environment
TNF-α
IL-1
IL-12
APC
(DC)
MHC-II
CD80/86
CD28/CTLA-4
CD40
CD4
TCR
CD40L
IL-4
IL-10
β
IL-1 TNF-α IFN-γ
Free radical
(O2-, NO)
MHC-I
CD8
Fas
FasL
TCR
perforin
Mø
Mø
Th1
Th1
Th1
Th2
IFN-γ
Tc
Tc
IL-2
genetic predispostion
C. Gysemans



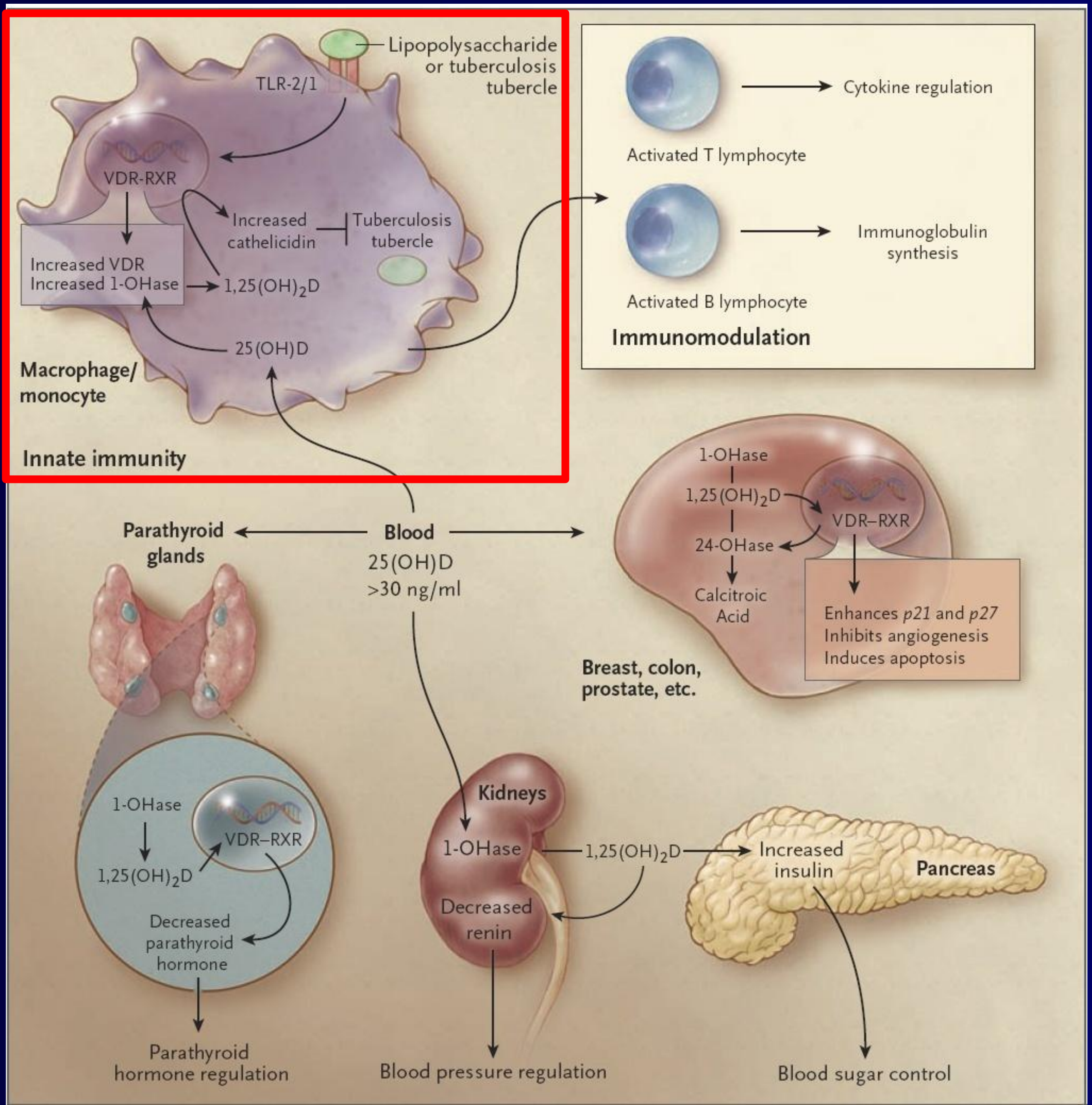
CHANTAL MATHIEU, U OF LEUVEN, 2009

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. Modlin[1,2]†

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.

HOLICK –Vitamin D Deficiency-NEJM 357:266-281 (July 19 2007)

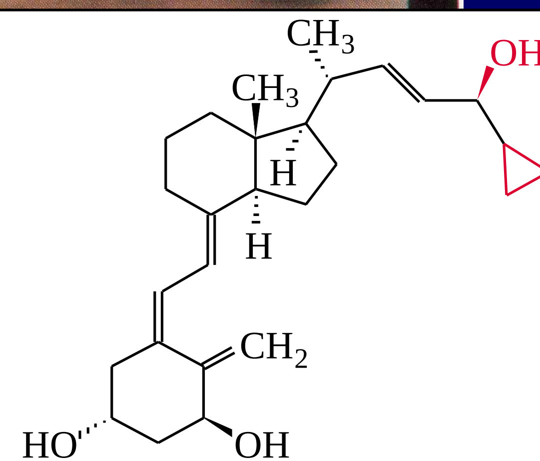


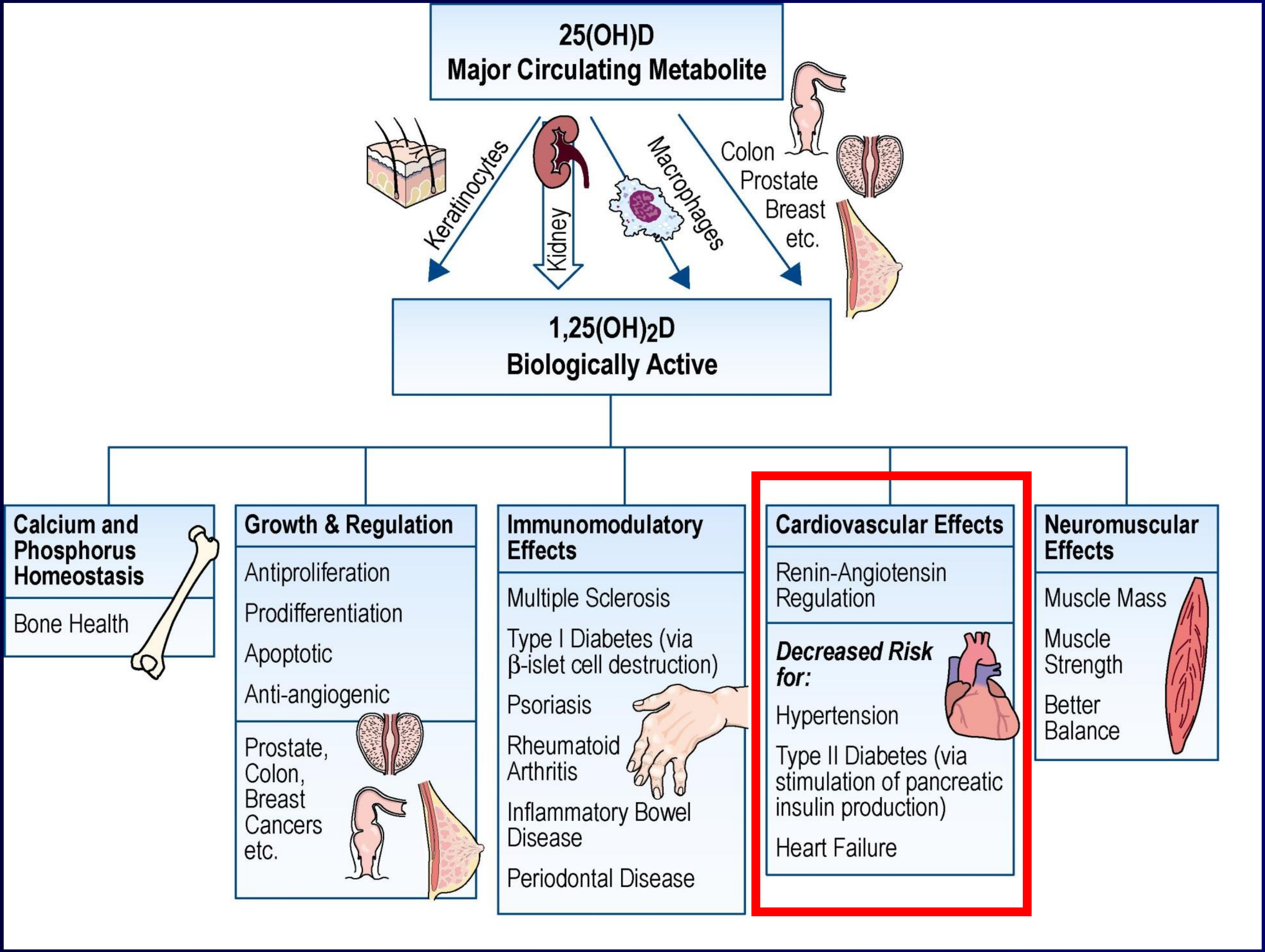
Evidence of a role for 1,25-$(OH)_2$D in Skin

LEO

Dovonex

calcipotriol

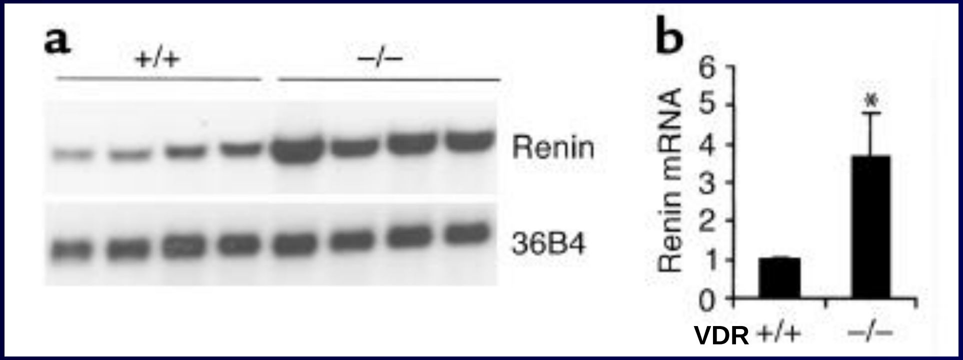


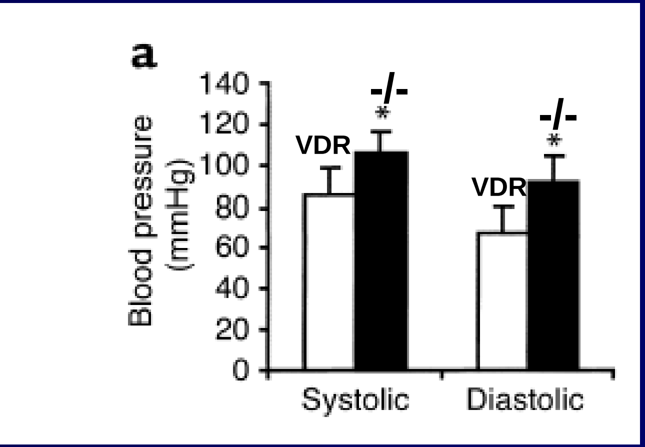

25(OH)D
Major Circulating Metabolite
Keratinocytes
Kidney
Macrophages
Colon
Prostate
Breast
etc.
1,25(OH)2D
Biologically Active
Calcium and Phosphorus Homeostasis
Bone Health
Growth & Regulation
Antiproliferation
Prodifferentiation
Apoptotic
Anti-angiogenic
Prostate, Colon, Breast Cancers etc.
Immunomodulatory 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

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**





Li, Yan Chun, Kong, J, The Journal of Clinical Investigation, 2002, Vol. 110, No. 2

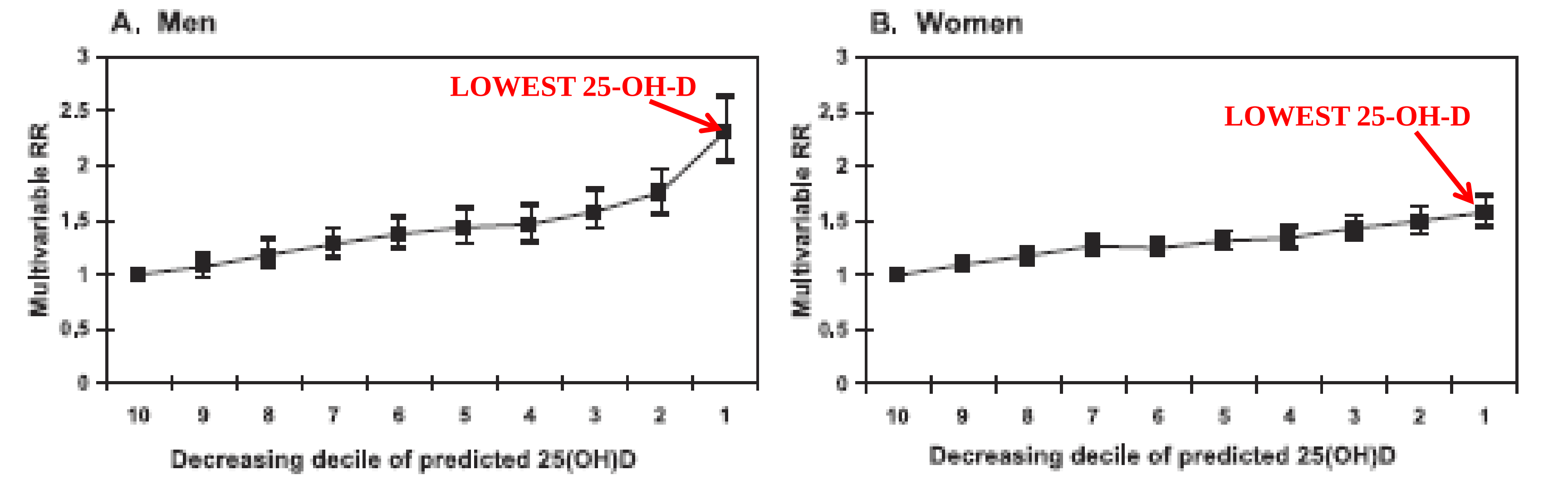
Vitamin D and Risk of Hypertension

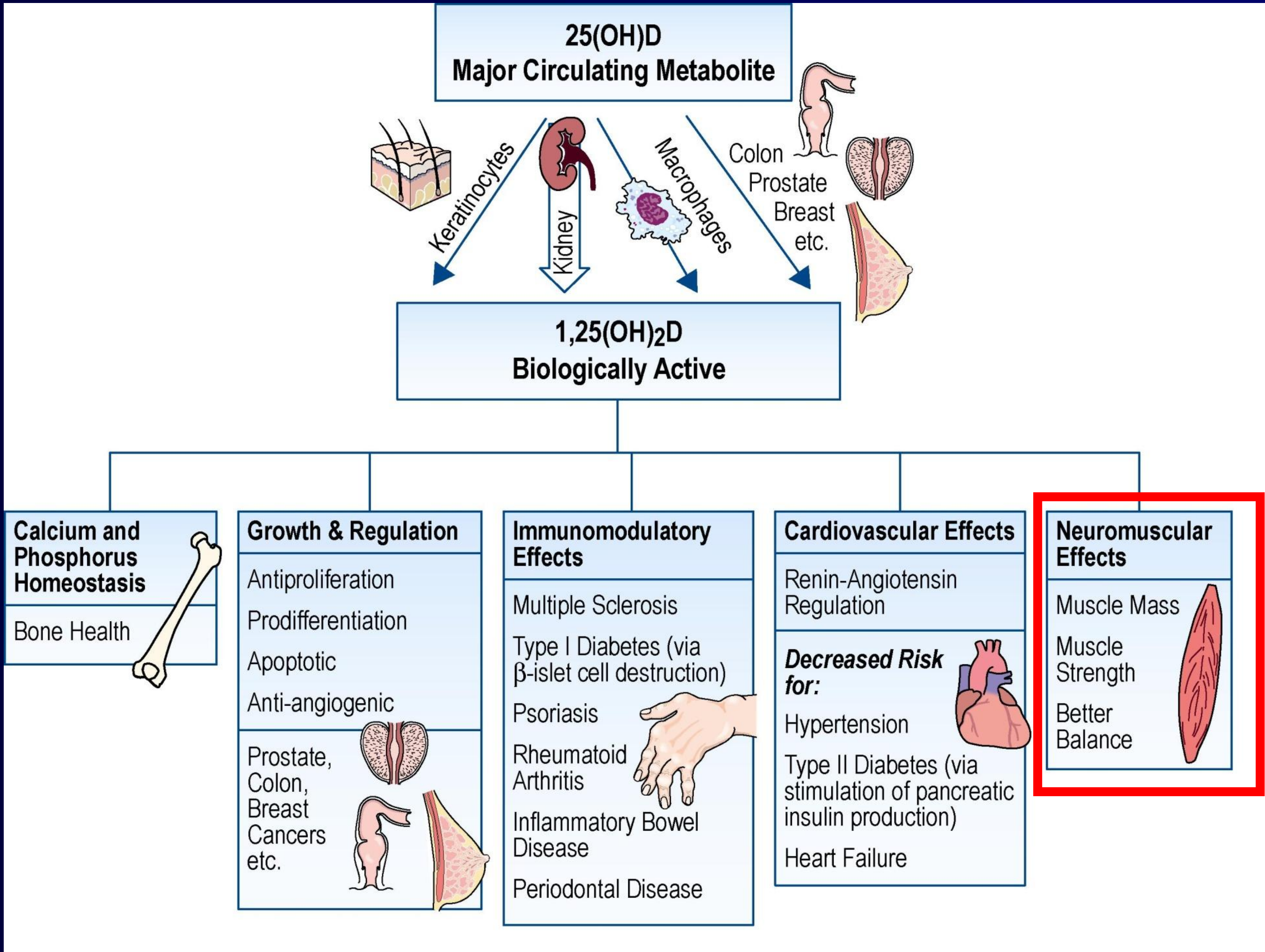
Plasma 25-Hydroxyvitamin D Levels and Risk of Incident Hypertension

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.**)

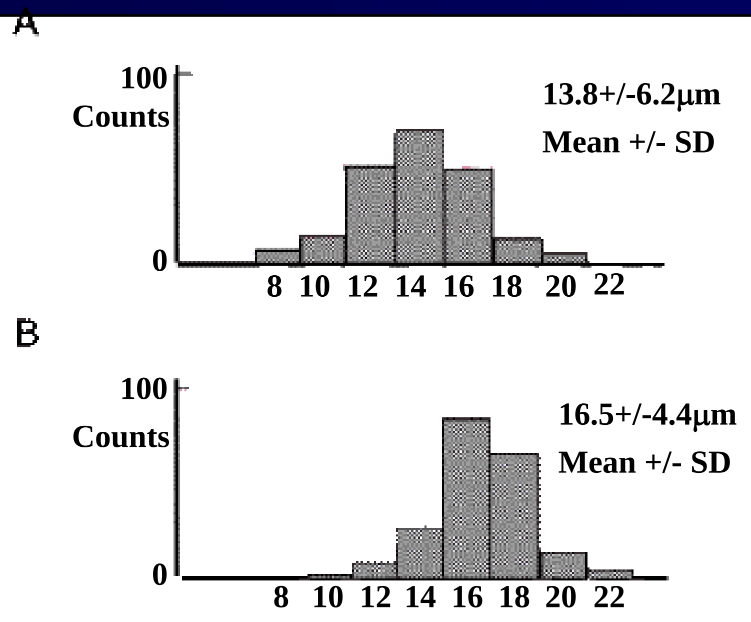
Key Words: vitamins ■ epidemiology ■ hypertension ■ risk factors ■ human




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

1,25-$(OH)_2$D & Muscle Differentiation

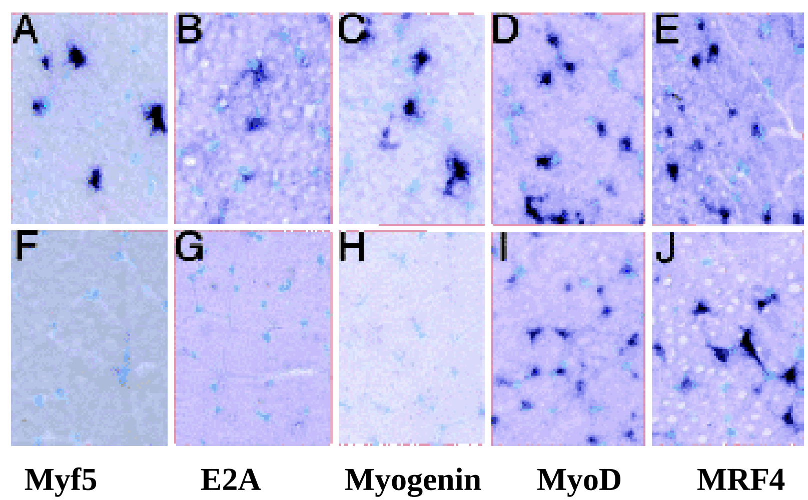
Muscle Fiber Diameter



VDR -/-

VDR +/+

Protein Expression



VDR -/-

VDR +/+

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

Prevention and treatment of vitamin D insufficiency and vitamin D deficiency in CKD patients.

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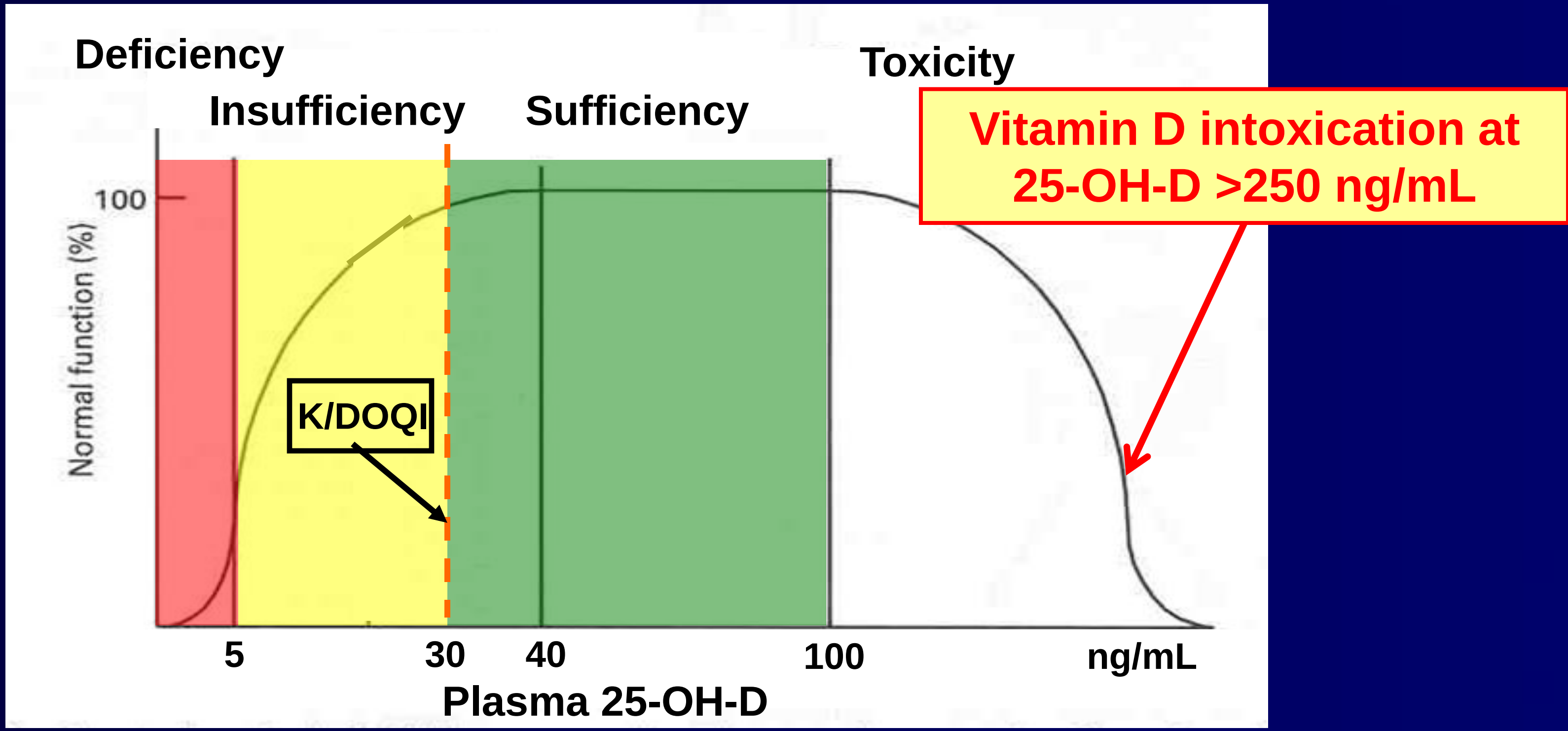
SUGGESTED THRESHOLD = 30 ng/mL or 75 nmol/L

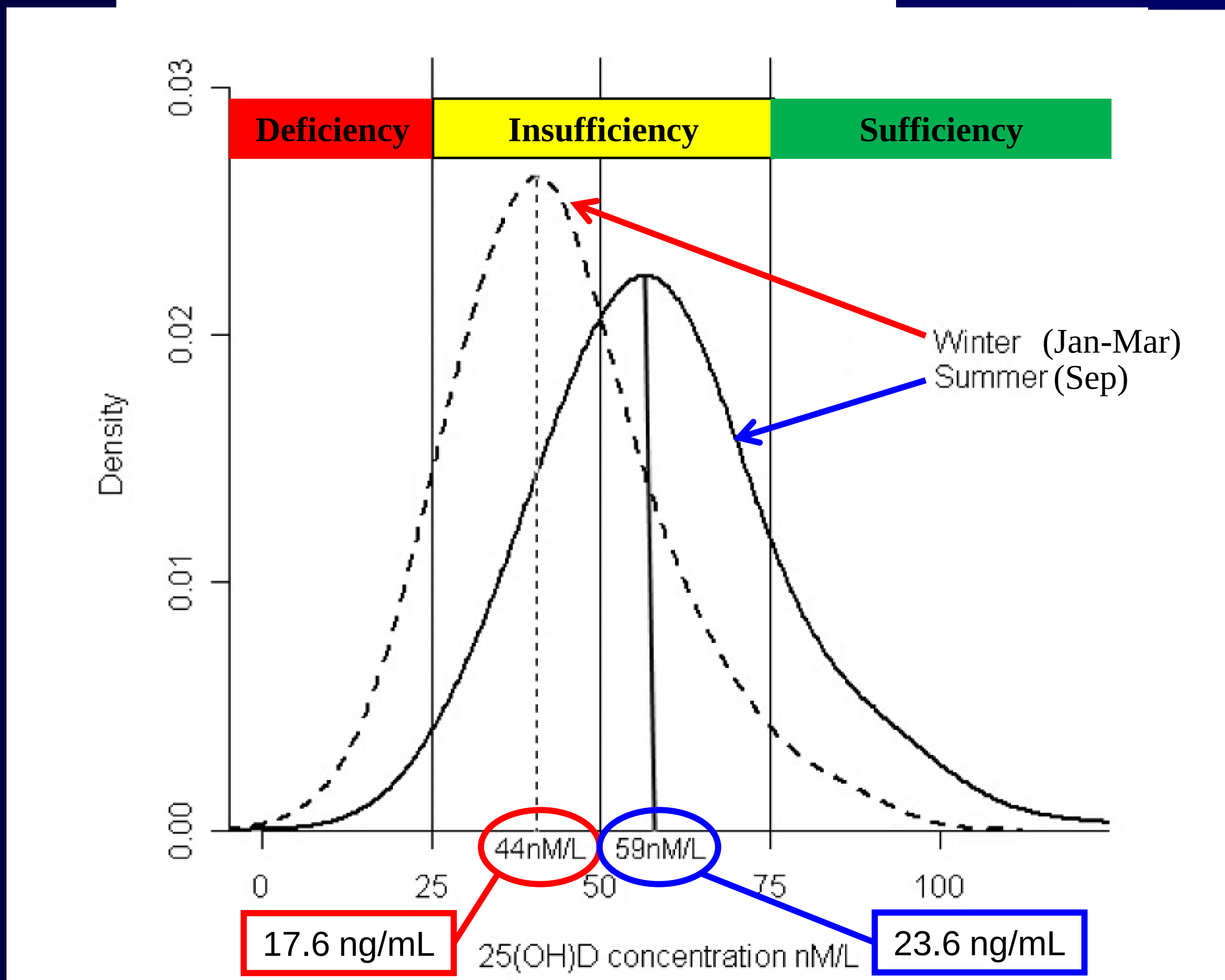


Adapted from Hollis

Seasonal variance of 25-(OH) vitamin D in the general population of Estonia, a Northern European country at Latitude 59º N

Mart Kull Jr*[1,2], Riina Kallikorm[1,2], Anu Tamm[2] and Margus Lember[1,2]

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**

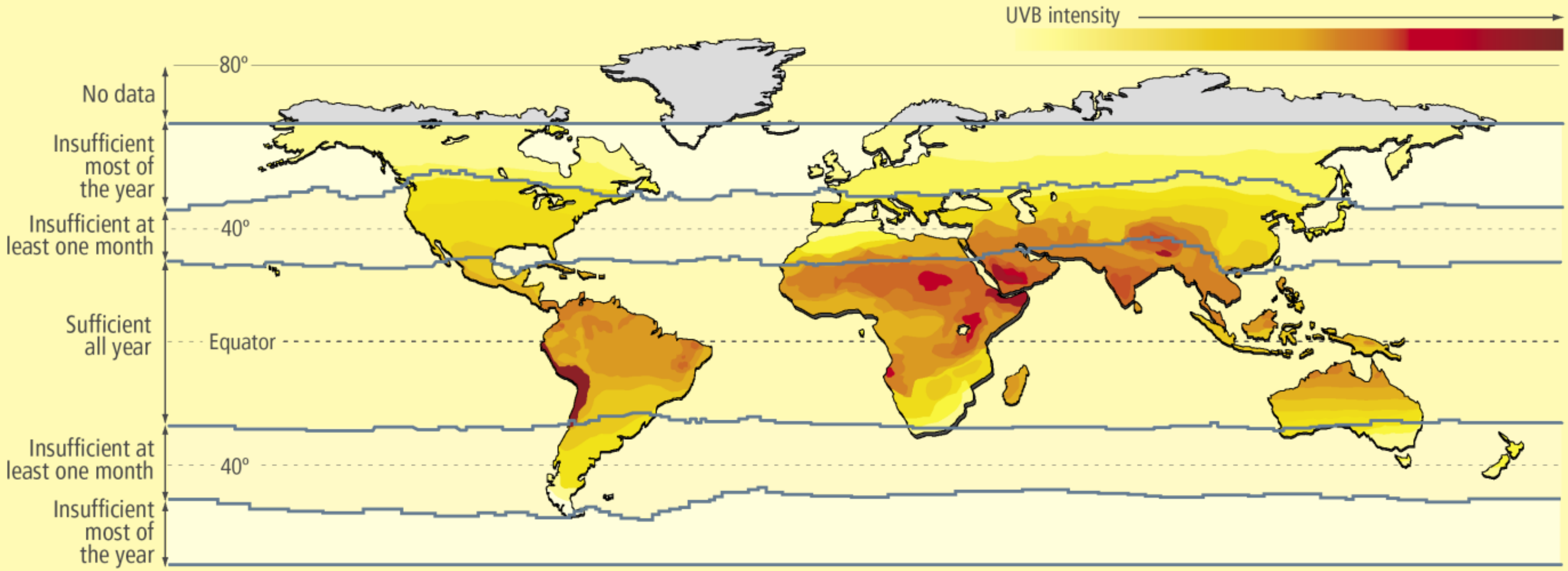
  - **PRESCRIPTION-VIT. D_2 in US (Drisdol)- 50,000 IU/dose**
  - **OTC VITAMIN PILLS-CURRENT DRI- 400-800 IU/day**
  - **OTC "DIETARY D_3 SUPPLEMENTS" IN USE- 1000-2000 IU**
  - **NATIONAL ACADEMY OF SCIENCE- NEW DRI-FALL 2010**

TAVERA-MENDOZA & WHITE, Sci Am pp 62-72 (Nov 2007)

[GLOBAL PROBLEM]

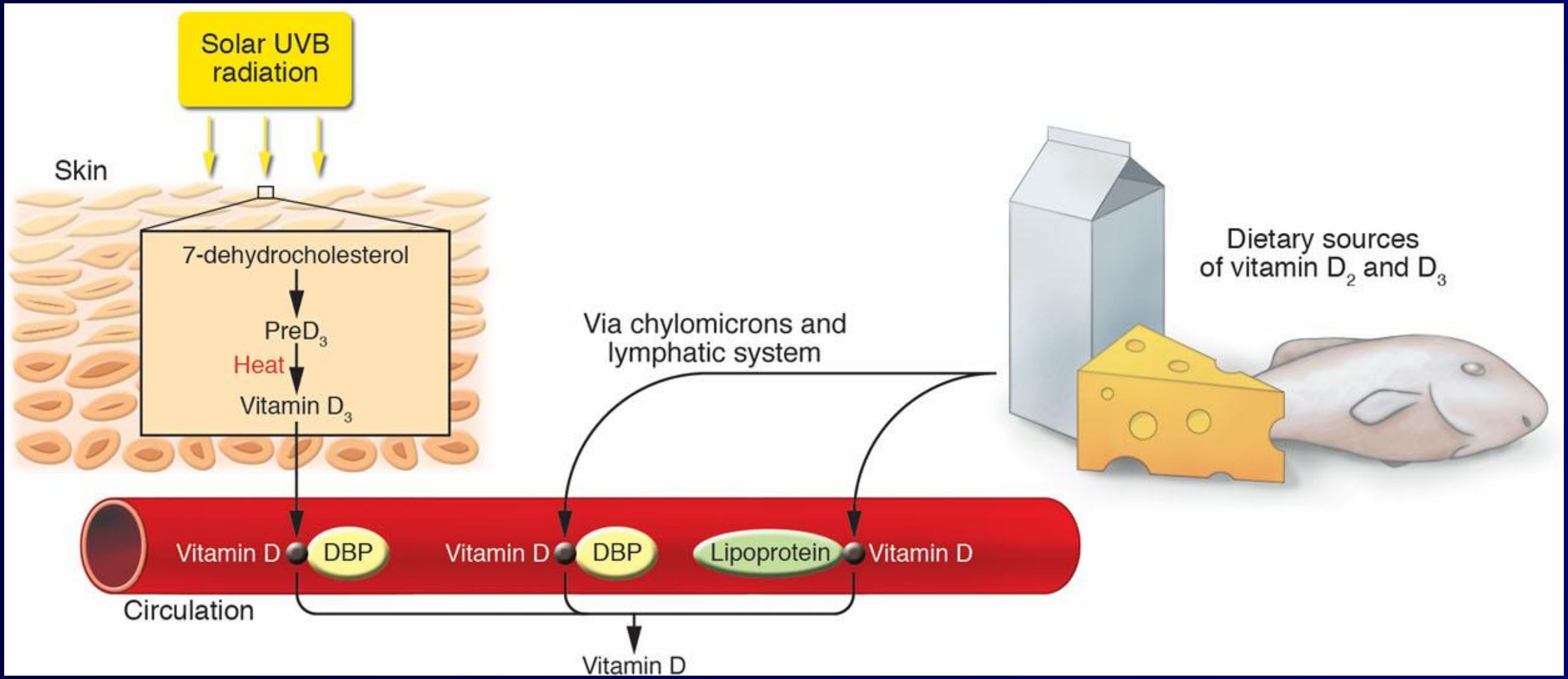
VITAMIN D WINTER

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
- $1\alpha,25\text{-}(OH)_2D$ has many varied classical and non-classical roles around the body
- Emergence of extra-renal 1α-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>

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