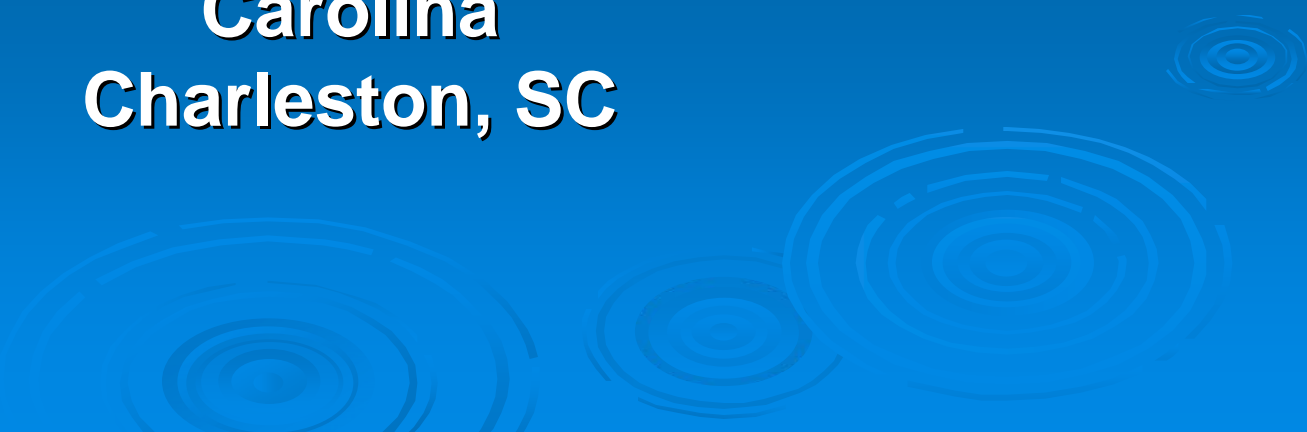


The Vitamin D Requirement During Pregnancy and Lactation

**Bruce W. Hollis, Ph.D.
The Medical University of South
Carolina
Charleston, SC**

The background of the slide is a solid blue color. In the lower right quadrant, there are several decorative elements consisting of concentric circles, resembling ripples in water. These circles are light blue and vary in size and opacity, creating a subtle pattern.

Disclosure

- Consultant to DiaSorin Corporation



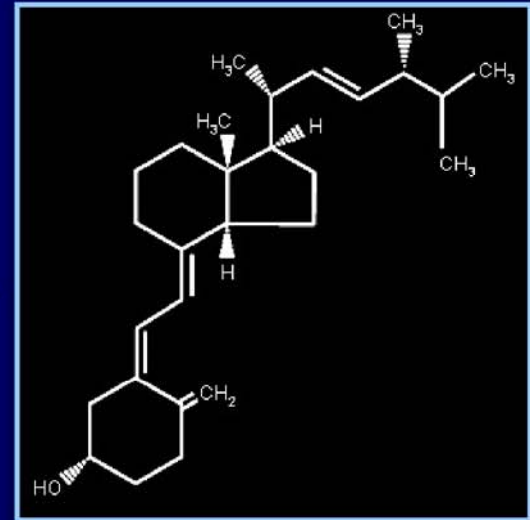
“Diagnosis and Treatment of Vitamin D Deficiency”

- Learning objectives:
- Understand what form of vitamin D to measure
- The desirable amount of vitamin D in your circulation
- How to obtain a desirable level of circulating 25(OH)D
- Consequences of suboptimal blood levels of 25(OH)D

Types of Vitamin D

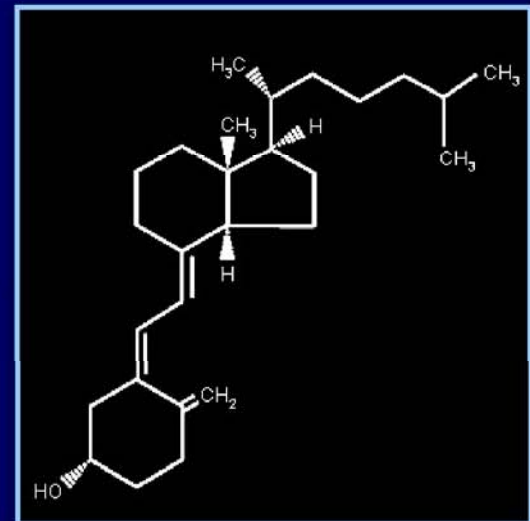
Vitamin D₂

- Formed by irradiation of ergocalciferol, found in plants
- Provided by some dietary sources and multivitamins
- Biologically inert
- Conversion (OH) in liver and kidneys produces active form
- D₂ is less potent than D₃



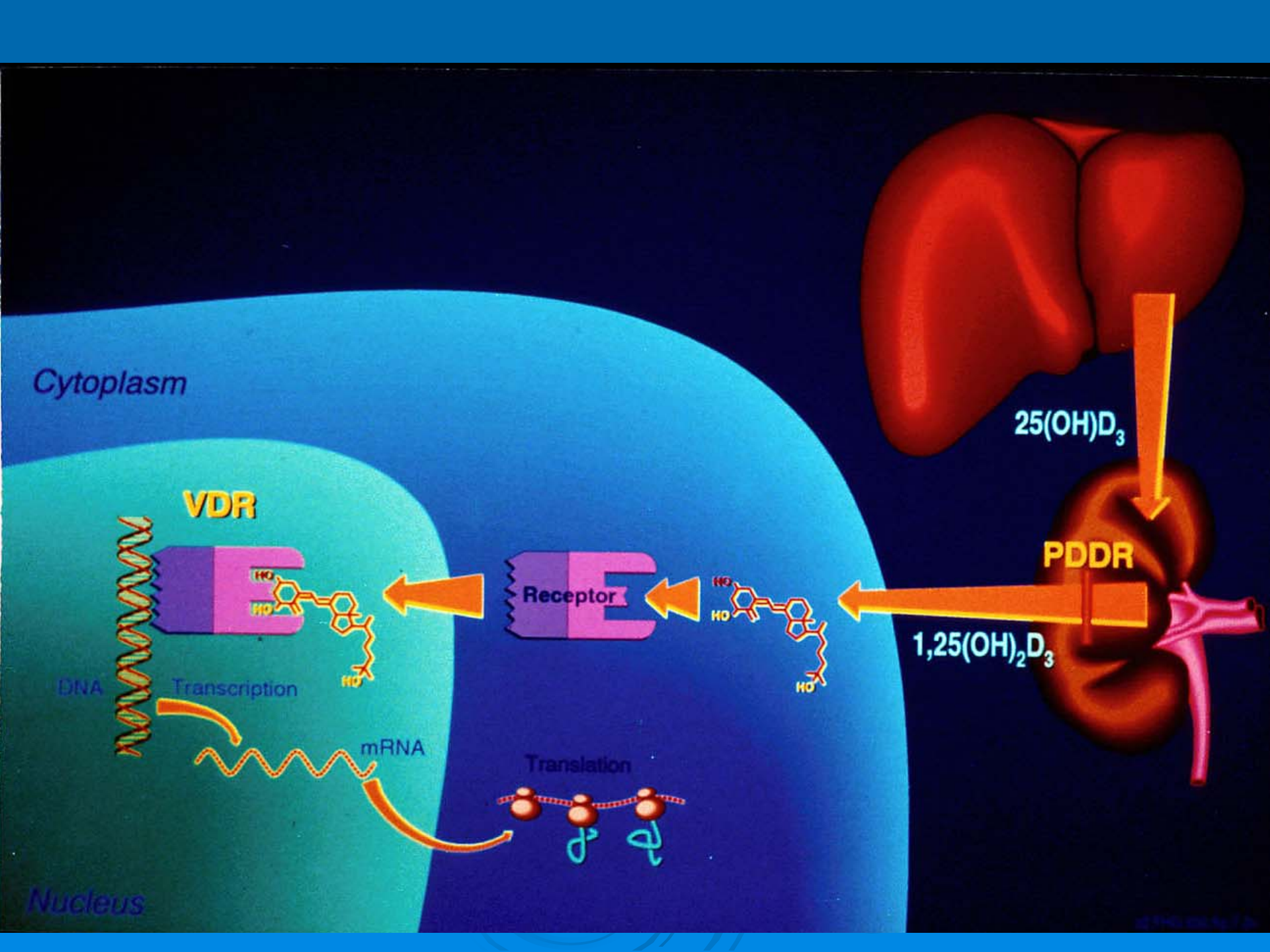
Vitamin D₃

- Naturally occurring form in humans
- Formed by action of ultraviolet light on vitamin D precursors in skin
- Present in certain nutrients
- Biologically inert
- Conversion (OH) in liver and kidneys produces active form



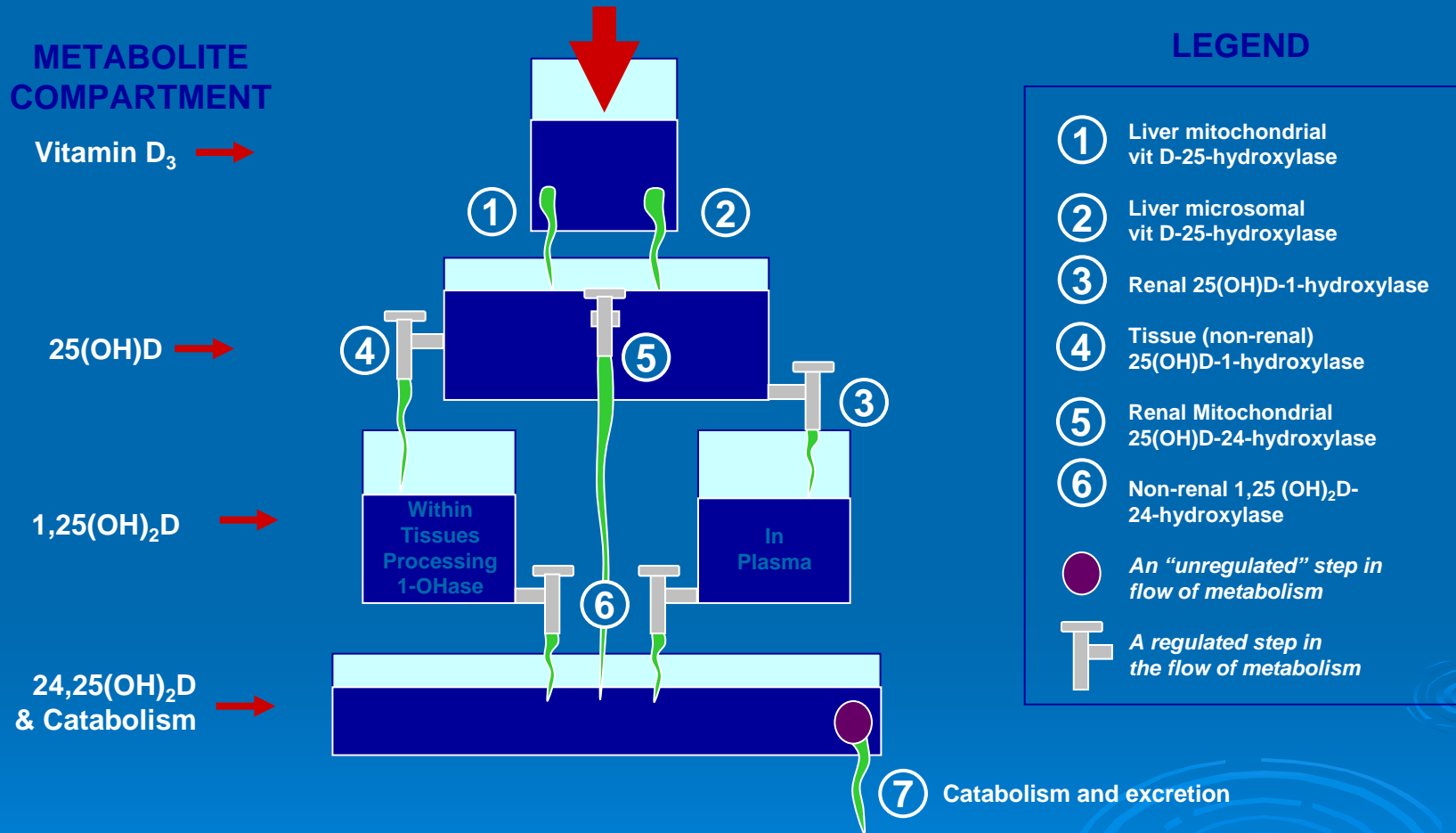
1. Parfitt AM et al. *Am J Clin Nutr.* 1982;36:1014–1031.

2. Lips P. *Endocr Rev.* 2001;22:477–501.



Metabolism of Vitamin D Under Conditions of Adequate Vitamin D Supply

High/Normal Input of
Cholecalciferol from diet or UVB



When vitamin D supplies are adequate, flow of 25(OH)D through other potential pathways, including its utilization by peripheral tissues for paracrine regulation, is no longer compromised.

Metabolism of Vitamin D Under Conditions of Low Vitamin D Supply

Low Input of Cholecalciferol from diet or UVB

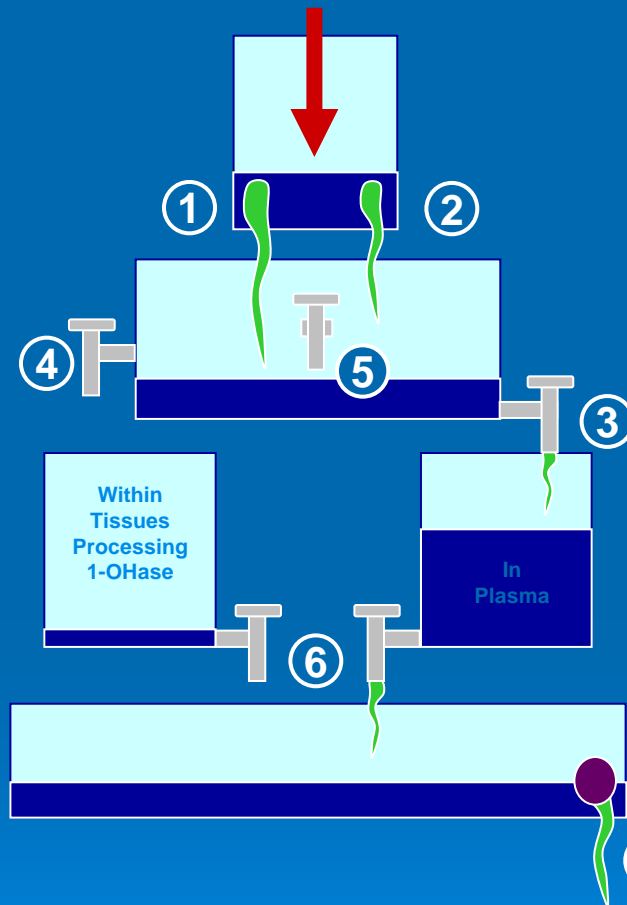
METABOLITE COMPARTMENT

Vitamin D₃ →

25(OH)D →

1,25(OH)₂D →

24,25(OH)₂D & Catabolism →



Legend

- ① Liver mitochondrial vit D-25-hydroxylase
- ② Liver microsomal vit D-25-hydroxylase
- ③ Renal 25(OH)D-1-hydroxylase
- ④ Tissue (non-renal) 25(OH)D-1-hydroxylase
- ⑤ Renal Mitochondrial 25(OH)D-24-hydroxylase
- ⑥ Non-renal 1,25 (OH)₂D-24-hydroxylase
- An "unregulated" step in flow of metabolism
- T A regulated step in the flow of metabolism

The vessels represent metabolic compartments, stages in the metabolism of vitamin D. The height of the shaded portion of each vessel represents the relative concentration of each metabolite indicated in the figure.

Substrate Deprivation

- The vitamin D endocrine system is the ONLY steroid endocrine system in the body that is almost always limited by substrate availability due to latitude, lifestyle, race etc.
- Vitamin D conversion to 25(OH)D.
- 25(OH)D conversion to 1,25(OH)₂D in extra-renal sites

Hypovitaminosis D among African American and White Women of Child-Bearing Age in NHANES III 1988-94

- **Prevalence of low vitamin D**
 - 42.4% in African American women
 - 4.2% in Caucasian women
- **African American women in urban environment more likely to have low vitamin D levels**
 - Odds ratio= 1.7 for low vitamin D levels
 - Current AI for vitamin D did little to correct the differences observed

Hypovitaminosis D during Pregnancy

- As currently defined (<80 nmol) , essentially 100% of women of color are vitamin D insufficient during pregnancy.

How is nutritional vitamin D deficiency defined?

Year	25 (OH) Vitamin D Level in plasma
1971-2003	<10-15 ng/mL (25-37.5 nmol/L)
>2003	25-32 ng/mL (70-80 nmol/L)

Hollis J Nutr (2005)

Hollis & Wagner CL.(letter to editor) Lab Ref Values. N Engl J Med (2005).

Laboratory Ref Data. Rochester, MN: Mayo Clinic, 2004.

The name “rickets” is from the Old English “wrickken”, to twist.





London 1889



Idaho 1989

5 mo: note "Hot X bun" skull



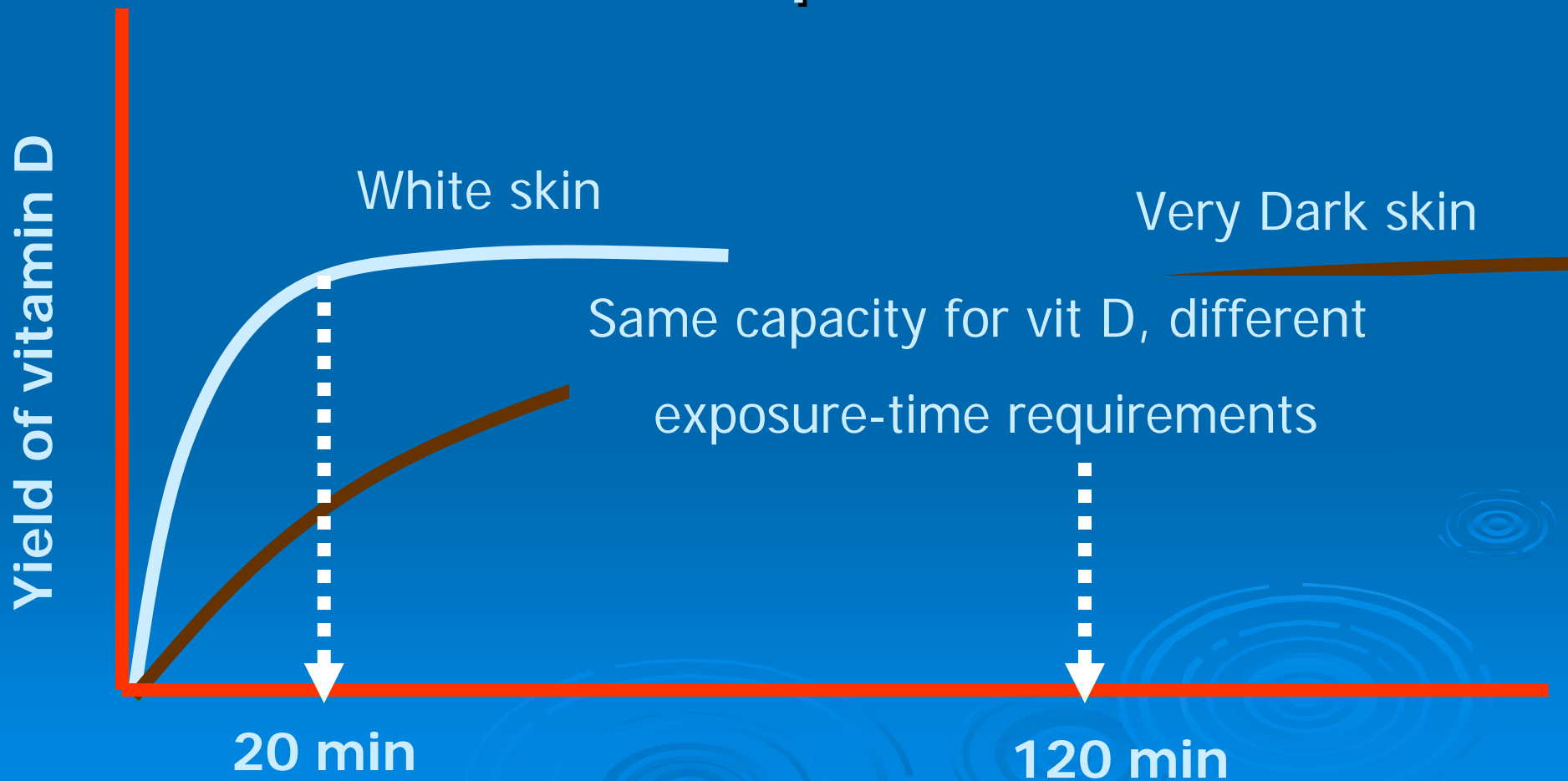


World Distribution of Nonhuman Primates

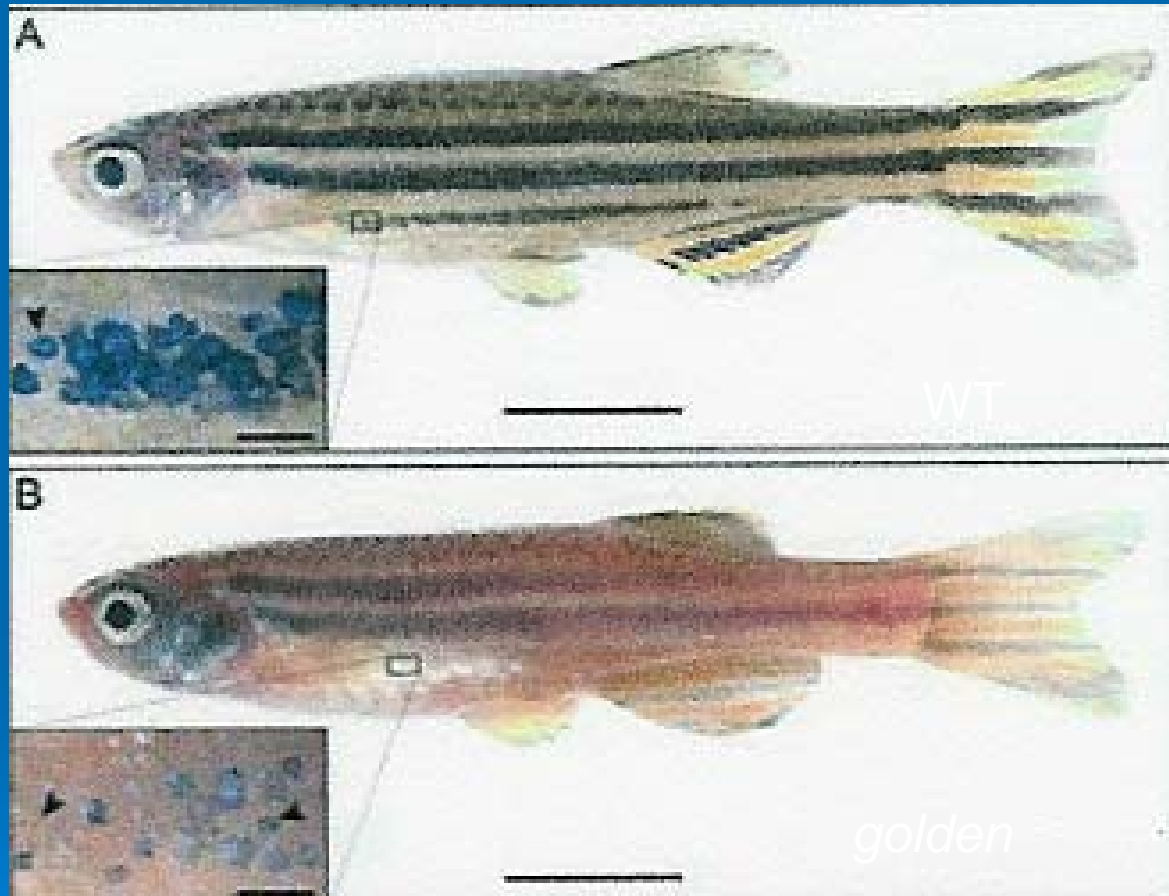


from; *Primate Behavior: Field studies of monkeys and apes.* I DeVore 1965

Effect of UVB exposure time and skin colour on Vitamin D production :

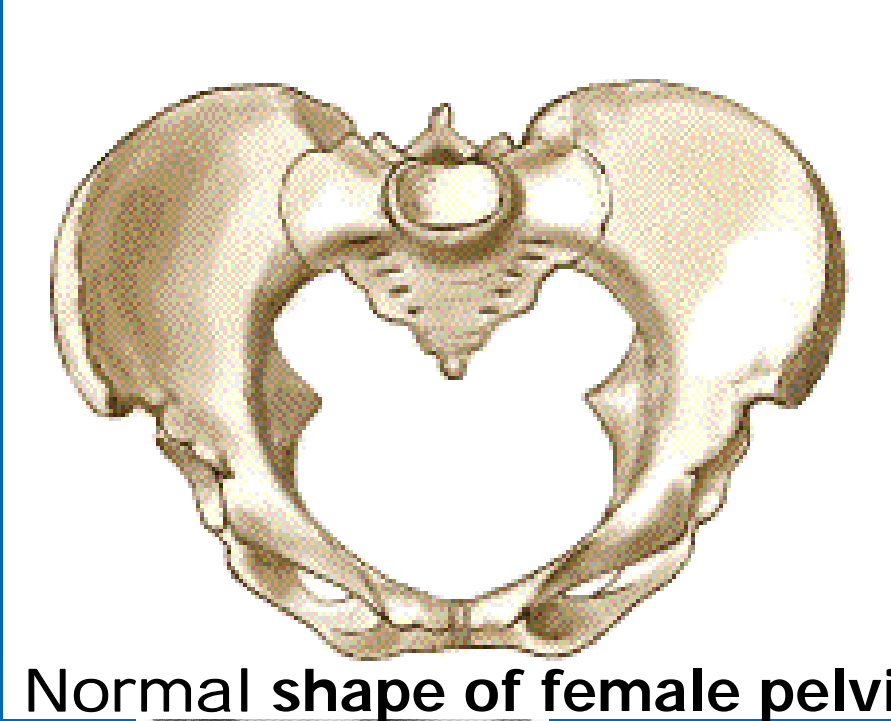


Phenotype of *golden* zebrafish

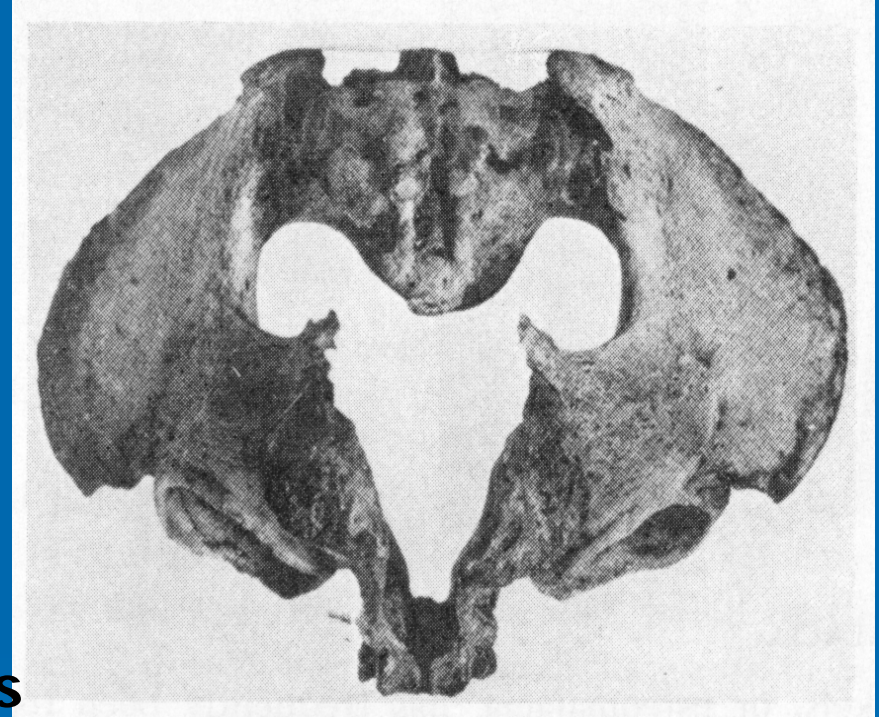


Lamason RL et al. Science 2005

Childhood lack of vitamin D causes rickets



Normal shape of female pelvis



Contracted pelvis, in a case of osteomalacia (adult rickets).

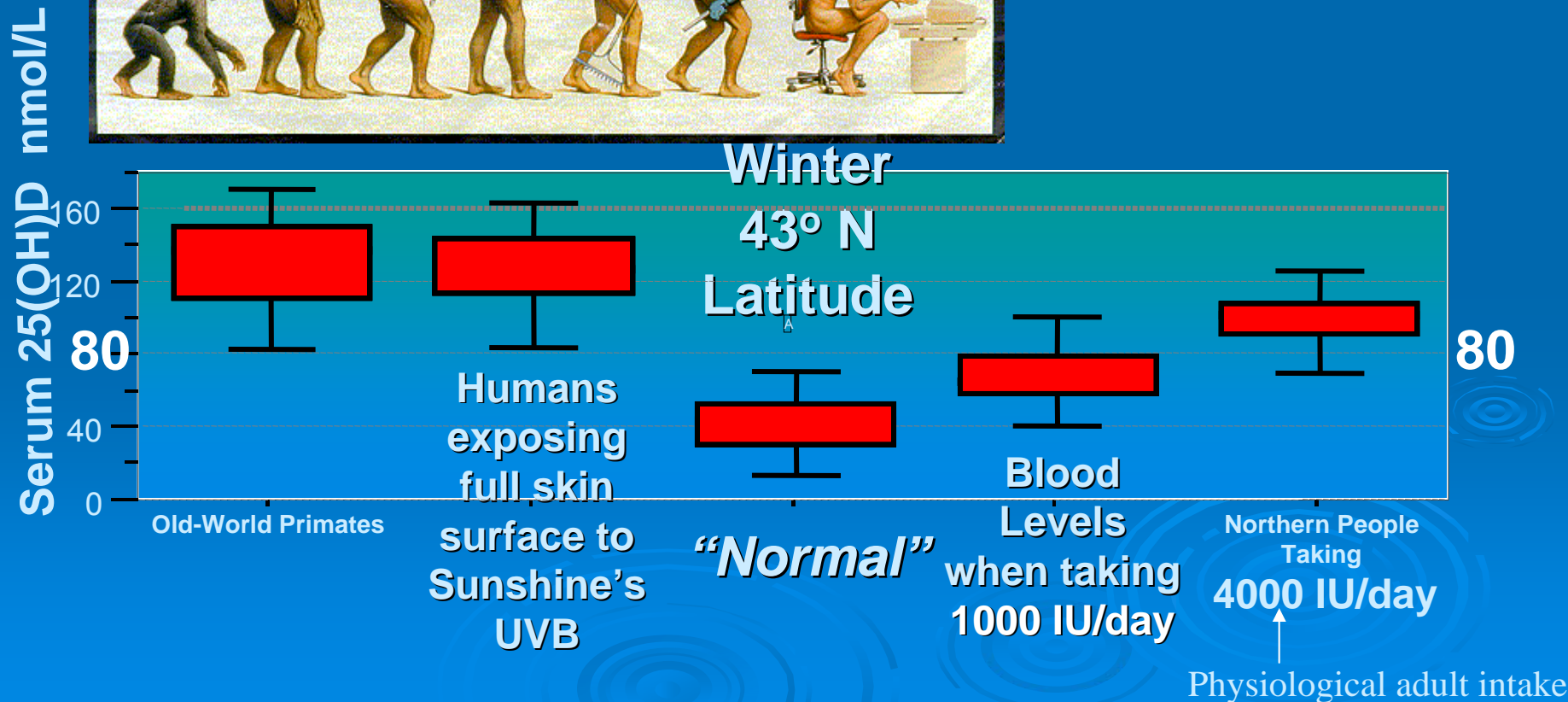
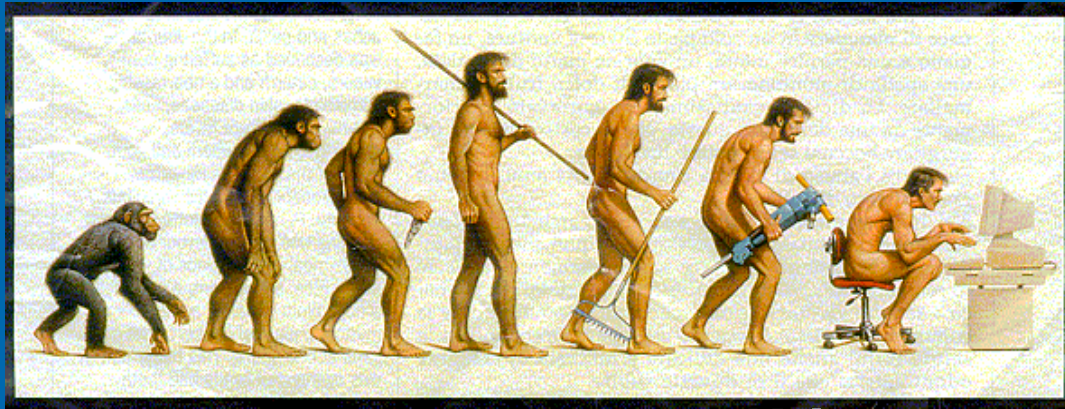
Normal childbirth would be impossible.



Optimal Circulating Concentrations of 25(OH)D



Vitamin D Status in Primates and Early Humans



Sources, include Cosman, Osteoporosis Int 2000; Fuleihan NEJM 1999; Scharla Osteoporosis Int 1998; Vieth AJCN 1999, 2000



QUEST DIAGNOSTICS INCORPORATED
JENKINS SERVICE 800.531.1199

ORDER INFORMATION
SPECIMEN: 48718128
ACQUISITION:

COLLECTED: 01/19/2007 13:00
RECEIVED: 01/19/2007 23:11
REPORTED: 01/23/2007 17:51

PATIENT INFORMATION

[REDACTED]

DOB: [REDACTED]
GENDER: F

ID: 11684
PHONE: [REDACTED]

REPORT STATUS **FINAL**

ORDERING PHYSICIAN

[REDACTED]

CLIENT INFORMATION
T59191 13080440
AFFILIATES IN CD/GYN
ATTN: REF. PHYSICIANS
121 ROUTE 31 SOUTH
FLORINGTON, NJ 08812

Test Name	In Range	Out of Range	Reference Range	Lab
CREATININE W/EGFR				TBR
CREATININE	0.7		0.5-1.2 mg/dL	
EGFR NON AFR AMERICAN	>60		>=60 mL/min/1.73m2	
EGFR AFRICAN AMERICAN	>60		>=60 mL/min/1.73m2	
PHOSPHORUS	3.1		2.5-4.5 mg/dL	TBR
VITAMIN D, 25-OH, LC/MS/MS				TBR
VITAMIN D, 25-OH, TOTAL	45		20-100 ng/mL	
VITAMIN D, 25-OH, D3	45		ng/mL	
VITAMIN D, 25-OH, D2	4		ng/mL	

25-OH(D3) indicates both endogenous production and supplementation.

25-OH(D2) is an indicator of exogenous sources such as diet or supplementation. Therapy is based on measurement of Total 25-OH(D), with levels <20 ng/mL suggesting Vitamin D deficiency while levels between 20 ng/mL and 30 ng/mL suggesting insufficiency. In both situations there is need for intense to moderate supplementation. In patients using D2 (ergocalciferol) supplementation, levels of 4 ng/mL of 25-OH(D2) or greater suggest compliance.

PERFORMING LABORATORY INFORMATION:

QR Quest Diagnostics One Malvern Avenue Tenaford NJ 07688 Laboratory Director: William E. Darr, M.D.
CLIA No. 1100490246

The Detection of Circulating 25(OH)D Levels

Lab Report Information Example

1.	25(OH)D	Total	21 ng/ml
2.	25(OH)D ₃		10 ng/ml
3.	25(OH)D ₂		11 ng/ml

Lab Range: 20-100 ng/ml

The TOTAL is what counts.

Labs need to update ranges to 40-100 ng/ml

Biomarkers for Vitamin D Sufficiency

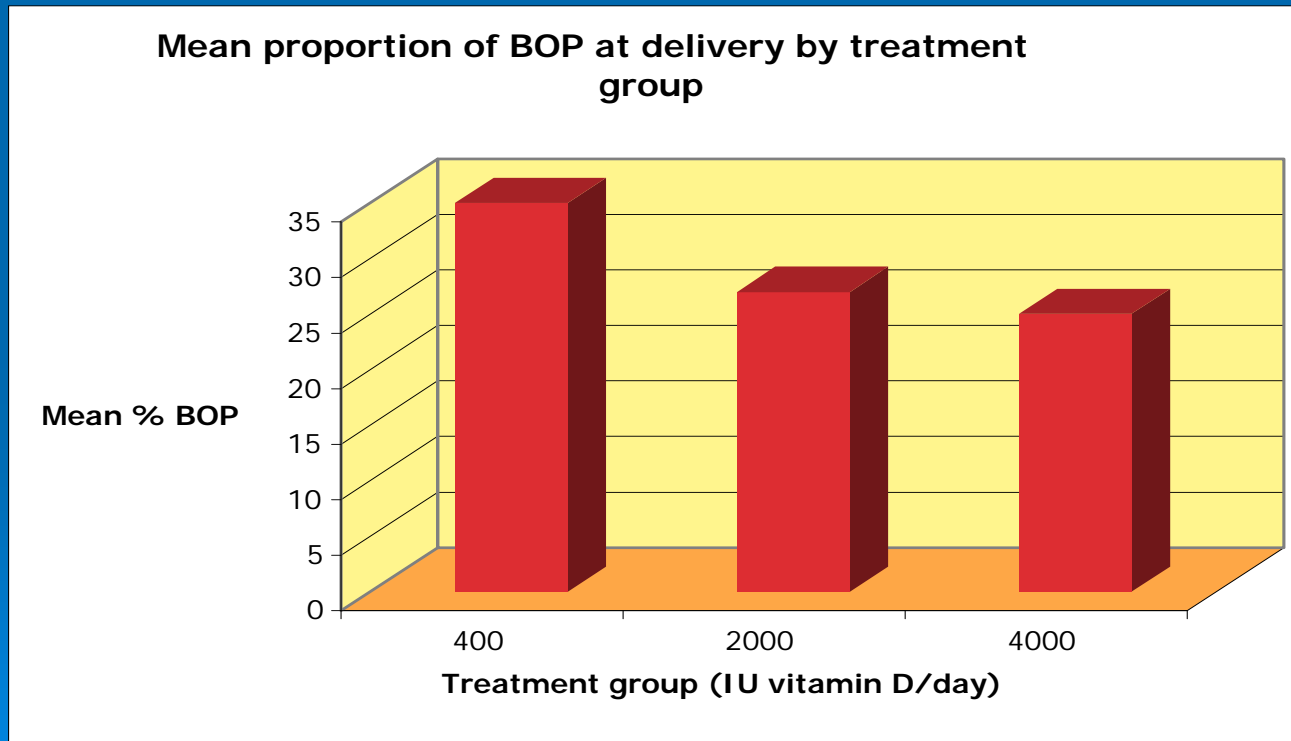
- 25(OH)D
- Intact PTH
- Bone Mineral Density (BMD)
- Intestinal Calcium Absorption
- Mobility responsiveness
- Insulin sensitivity
- Beta cell function
- Immune function
- Cardiovascular function

Should Vitamin D Supplementation During Pregnancy Be Of Concern?

- **Concerns about calcium homeostasis and skeletal integrity**
- **Does “imprinting” with low prenatal vitamin D contribute to the risk of various adult disorders?**
- **Hypovitaminosis D is a candidate risk-modifying factor for a diverse range of disorders apart from rickets and osteoporosis.**
 - Multiple sclerosis**
 - Increased cancer rates (13 types)**
 - Type I diabetes**
 - Schizophrenia**
 - Immune capacity (both innate and adaptive)**

Results: Bleeding on Probing

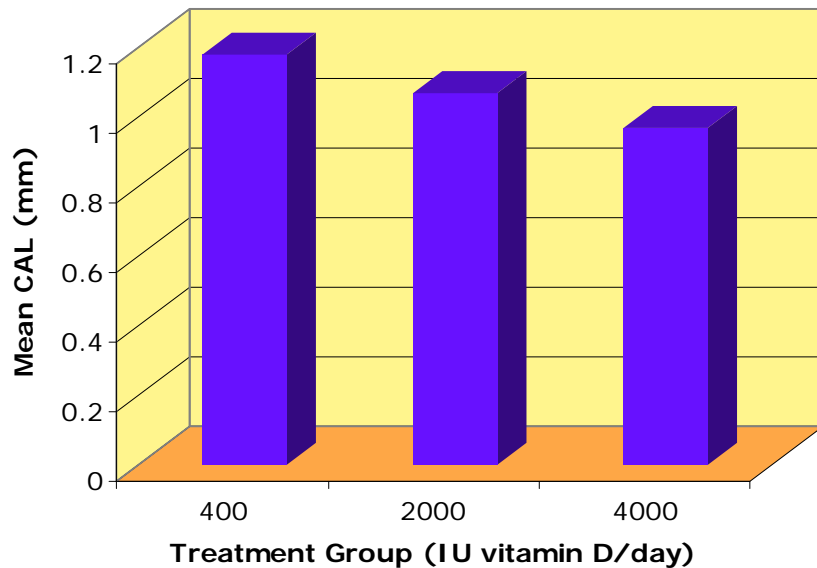
- Trend analysis done on BOP shows a significant difference among both treatment groups ($p=0.01$)



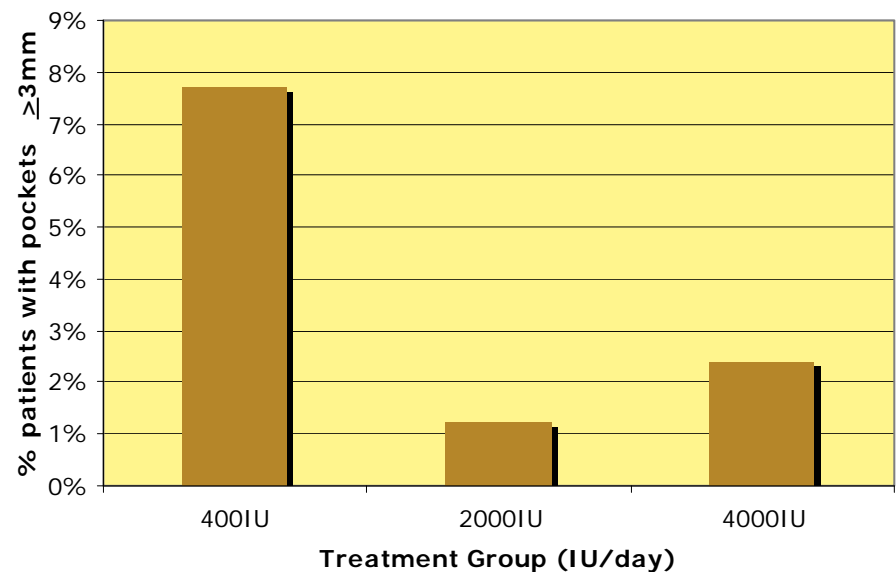
Results: Clinical Attachment Level

- Regression analysis done on CAL shows significant reduction from control to 4000 IU treatment group
- Clinically significant attachment loss: It appears that Vitamin D supplementation reduces the proportion of individuals with CAL ≥ 3

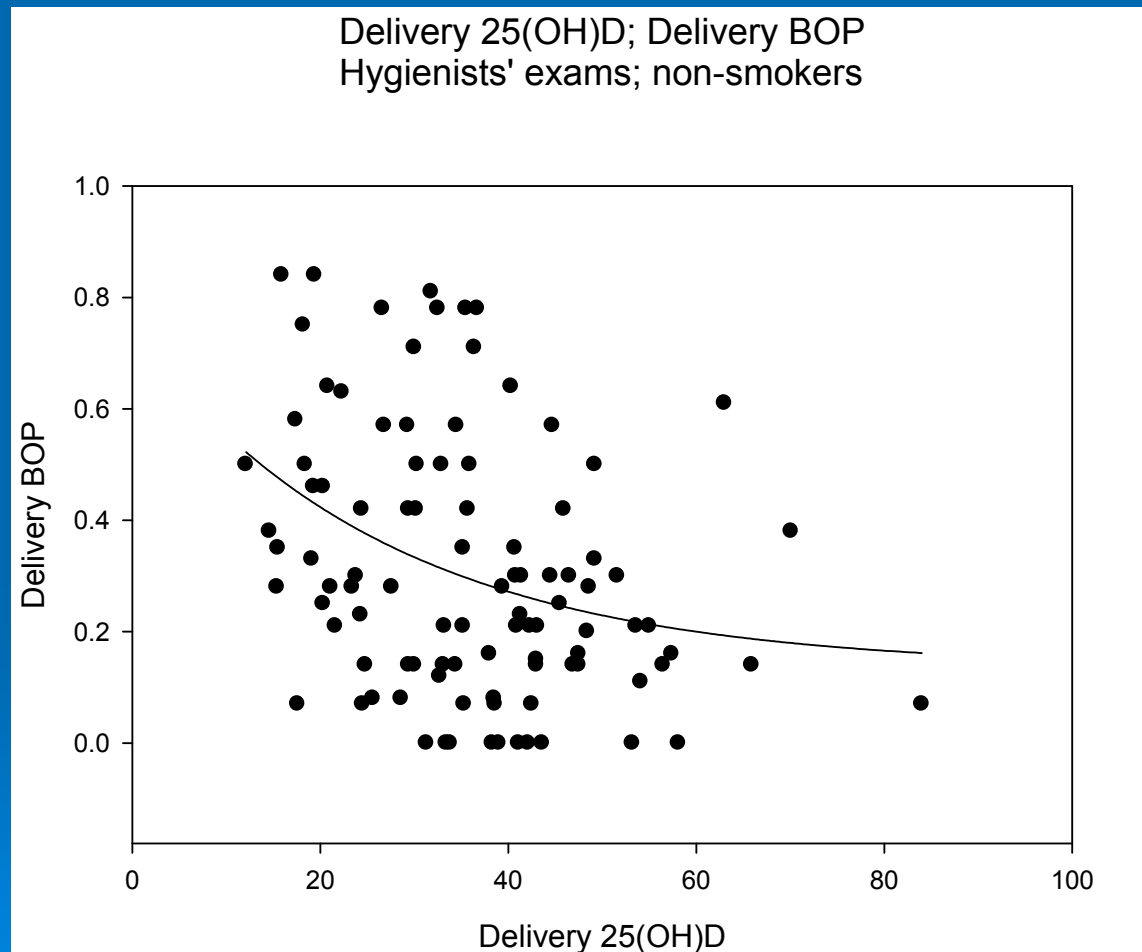
Mean CAL at Delivery by Treatment Group



Clinical Significance of Attachment Level



Gingival inflammation (percent of sites with bleeding on probing) by serum 25(OH)D levels at delivery in non-smokers



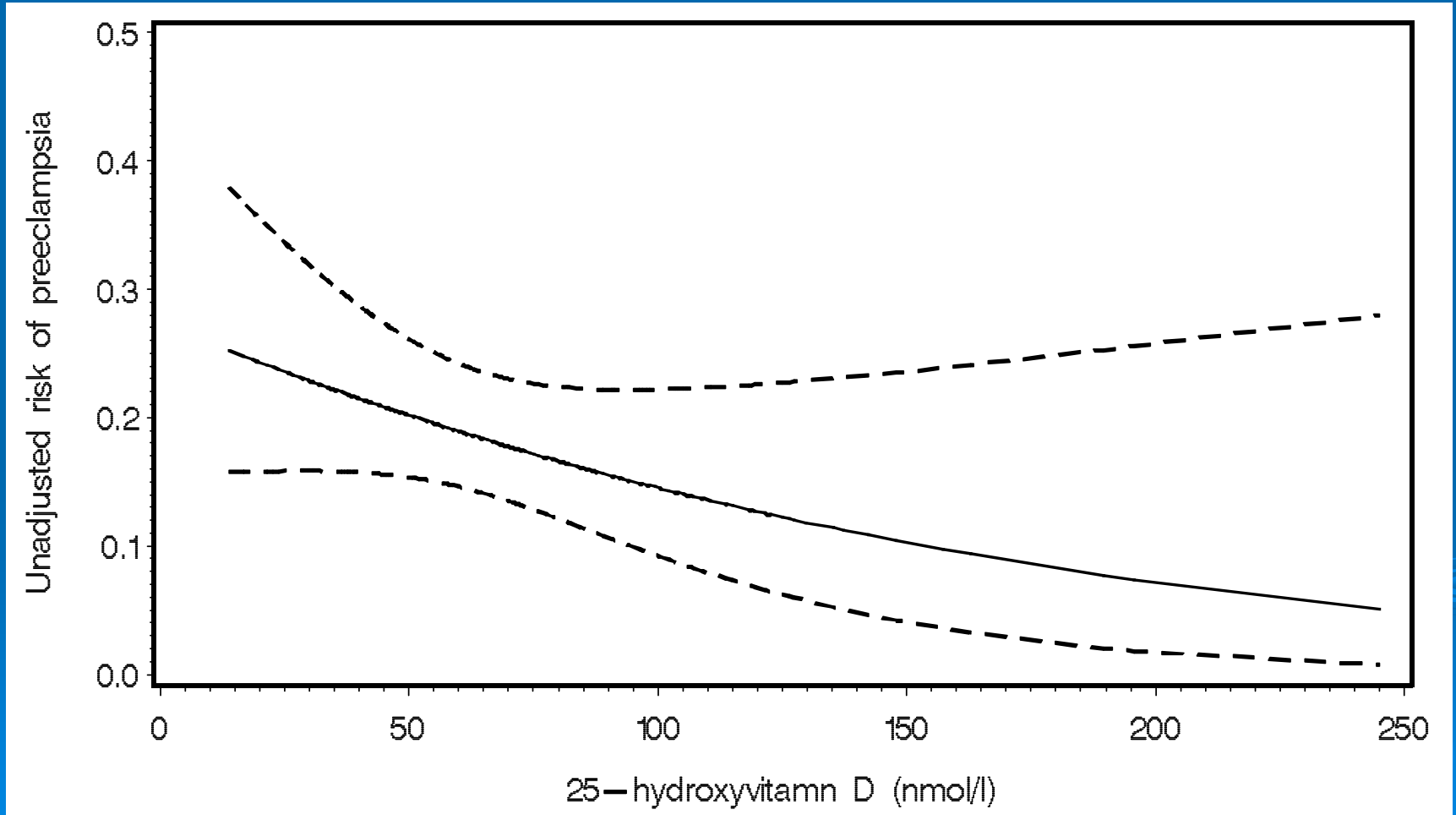
Point of inflection: 25(OH)D = 37 μ g/L
 $R^2 = 0.1$, $p < 0.0001$

Vitamin D deficiency was more common in women with preeclampsia than in controls

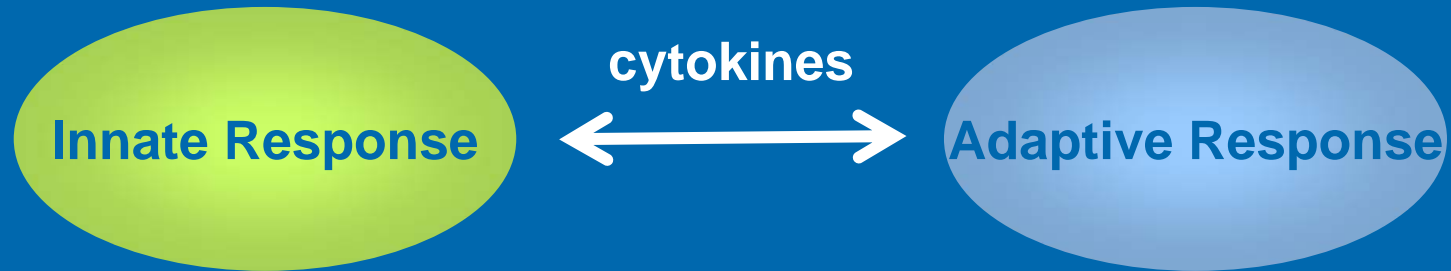
25(OH)D at <22 weeks	Control n	Preeclampsia n	Adjusted* OR (95% CI)
<37.5 nmol/l	27	35	5.0 (1.7, 14.1)
≥37.5 nmol/l	152	14	1.0 (ref)

*Adjusted for race/ethnicity, season, sample gestational age, prepregnancy BMI, and education.

Strong, inverse relation between maternal 25(OH)D at <22 weeks and risk of preeclampsia

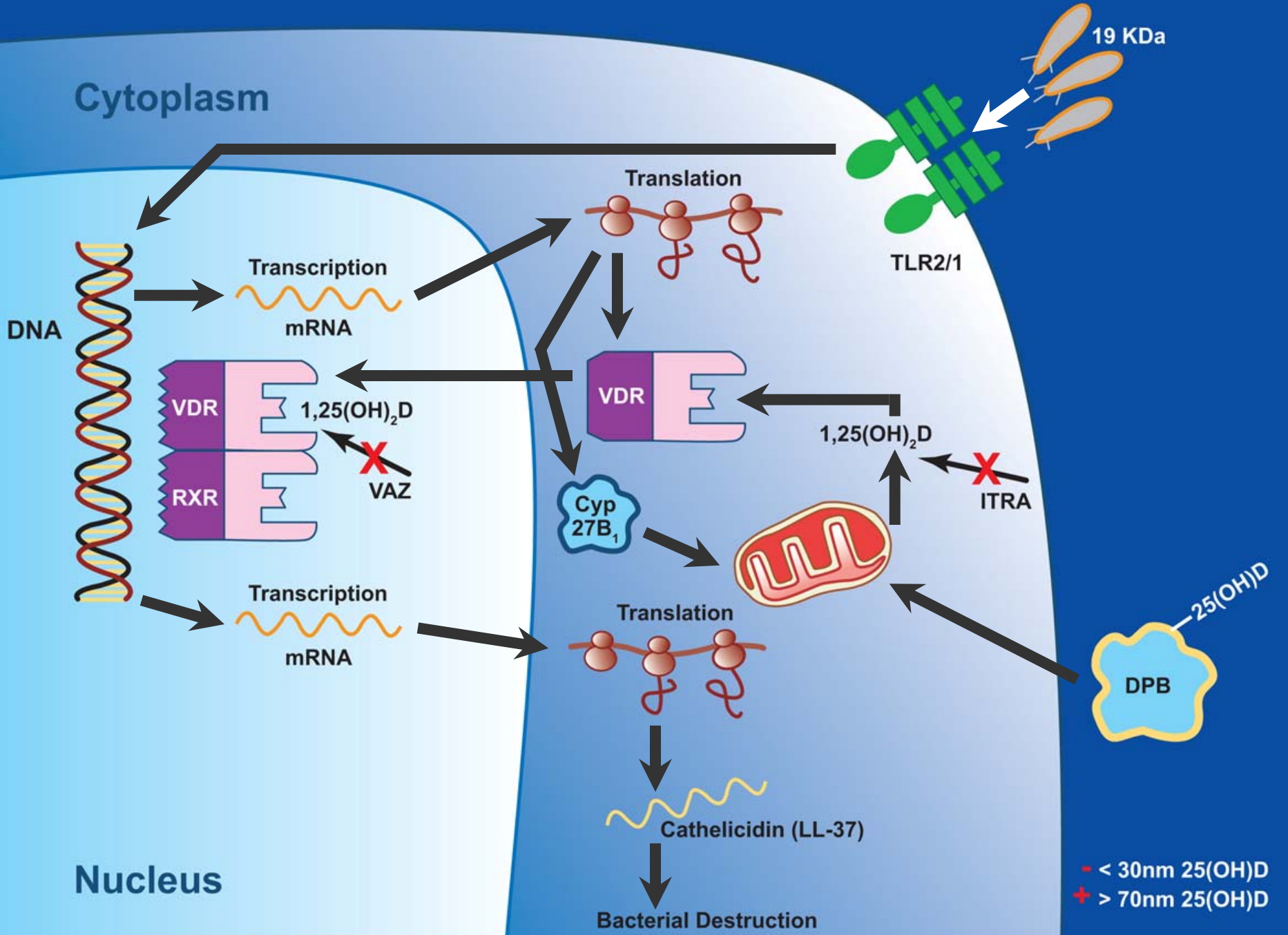


INNATE AND ADAPTIVE IMMUNITY



- Rapid response
- Pattern recognition receptors- germ-line encoded
 - LPS, mannose and scavenger
- ↑ Cytokines, costimulatory molecules- instructive role for adaptive response
- Direct response for host defense
 - Phagocytosis
 - Antimicrobial activity

- Slow response
- Recognition - initially low affinity receptors
 - Gene rearrangement
 - Clonal expansion
- Response: T- and B-cells with high affinity, very specific receptors and antibodies
- Memory



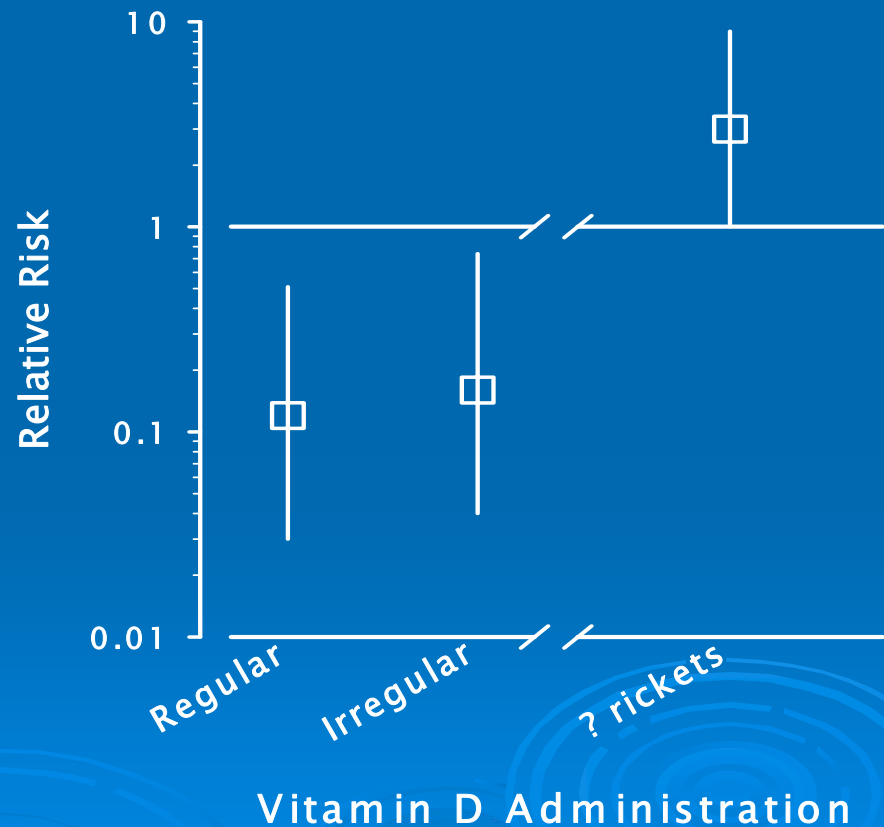
Serum 25-Hydroxyvitamin D Levels and Risk of Multiple Sclerosis

KL Munger, LI Levin, BW Hollis, NS Howard, A Ascherio

- **Objective:** To evaluate whether serum levels of 25(OH)D predict risk of MS among healthy adults
- **Methods:** Prospective study among >7 million US military personnel with ≥ 1 serum sample stored in Dept of Defense Serum Repository (DoDSR).
 - MS cases identified from Army and Navy Physical Disability Agencies and medical records reviewed to confirm dx using Poser criteria.
 - Each case was matched to 2 controls on age, sex, race, and dates of blood collection.
 - 25(OH)D measured using RIA in up to 3 samples collected prior to onset of first MS s(x).
- **Results:** 305 individuals with MS and 610 controls included in study.
 - Average age at MS onset was 28 years; 57% of cases and controls were White, 30% Black.
 - Elevated 25(OH)D levels in Whites more protective at younger ages.
 - **Individuals <20 years old with average 25(OH)D ≥ 100 nmol/L had 91% reduction in MS risk compared to those with average 25(OH)D <100 nmol/L (RR=0.09, 95%CI:0.01-0.78, p=0.03).**
 - JAMA 296, (2006)

NEONATAL VIT D & DIABETES*

- 10,366 northern Finnish children
- 2000 IU Vit D/d 1st year of life
- prevalence of type I diabetes assessed at age 21
- RR calculated vs. no supplementation



*Hypponen et al., Lancet 2001;358:1500-03

Cochrane Review

- **Assessed effects of vitamin D supplementation in acceptably controlled trials during pregnancy**
- **Identified 4 acceptable trials**
 - **Brooke et al (1980) studied British mothers of Asian decent receiving placebo or 1,000 IU/d vitamin D₂ during last trimester of pregnancy.**
 - Neonates in placebo group exhibited more SGA and greater fontanelle area.
 - There was rampant hypovitaminosis D in the placebo group.
 - **Brooke et al (1981) in a follow-up study demonstrated that infants from placebo mothers gained less weight and exhibited decreased linear growth following birth.**

Cochrane Review (con't)

- **Cockburn et al (1981) undertook a study in The UK providing > 1,000 women placebo or 400 IU/d vitamin D₂ or the last two trimesters of pregnancy.**
 - Circulating 25(OH)D levels were similar between groups with a possibility of defective enamel formation in placebo group at three years of age.
- **Maxwell et al (1981) conducted a double-blind trial giving 1,000 IU/d vitamin D₂ during the last trimester of pregnancy in Asian women in London.**
 - This study found supplemented mothers had greater weight gain, increased serum RBP and thyroid-binding prealbumin.
 - Infants in control group also exhibited lower circulating RBP levels.

Other Studies

- **Brunvand et al (1996) followed 30 pregnant Pakistani women who were free from chronic disease and had uncomplicated pregnancies.**
 - Nearly all of the women exhibited 25(OH)D levels < 15 ng/ml and $> 50\%$ demonstrated hyperparathyroidism.
 - Maternal circulating PTH was inversely related to neonatal crown-heel length indicating that maternal vitamin D status may be linked to fetal growth.
- **Datta et al (2002) provided pregnant minority women in the UK with 800-1,600 IU/d vitamin D₂ throughout their pregnancy.**
 - Circulating 25(OH)D increased from 5.8 \rightarrow 11.2 ng/ml during gestation.
 - This was a PATHETIC increase in this group of subjects.

Summary From Cochrane

- There is not enough evidence to evaluate the effects of vitamin D supplementation during pregnancy.

**Vitamin D as a cause of the
supravalvular aortic stenosis syndrome**

William F. Friedman, M.D.
Cardiology Branch
National Heart Institute

Am. Heart J.
May, 1967

Evaluation of the Vitamin D Requirement During During Pregnancy

NIH #5R01HD043921-04

Specific Aim 1

- **Determine the efficacy, effectiveness and safety of prenatal maternal vitamin D supplementation as a function of ethnicity and *UV* exposure in the prevention of hypovitaminosis D**

Hypotheses for Specific Aim 1

- Hypothesis 1: Prenatal maternal vitamin D requirement is greater in darkly pigmented pregnant women due to limited synthesis of vitamin D₃ in the skin.
- Hypothesis 2: High-dose (2,000 or 4,000 IU/day) vitamin D₃ supplementation of pregnant mothers will be a safe supplement for mother and fetus and provide sufficient antirachitic activity to prevent hypovitaminosis D in the pregnant mother and her fetus, without regard to race and sunlight exposure.

Specific Aim 2:

- Determine the efficacy and effectiveness of prenatal maternal vitamin D Supplementation on neonatal and infant skeletal integrity and growth.
 - *Hypothesis:* High-dose prenatal vitamin D supplementation will result in enhanced infant skeletal integrity and growth.

Study Design

- 5-Yr Prospective, randomized, double-blind placebo-control vitamin D supplementation trial

Pregnant women stratified by ethnicity/race

African American (n=180)

Caucasian (n=180)

Hispanic (n=180)

- Each ethnic/racial group randomized to one of three vitamin D doses from 12 weeks gestation → term.

- 60 women in each subgroup
- Oral vitamin D doses for mother
 - 400 IU/day (control)
 - 2,000 IU/day
 - 4,000 IU/day

Study Progress to Date

- IRB approval February 2003
- IND granted on May 2003
- NIH Grant funding September 2003
- Radiation Safety approval December 2003
- First subjects enrolled January 2004
- >500 completed or active patients without a single adverse event linked to vitamin D intake.

Vitamin D Supplementation During Lactation

- Scientific data pertaining to vitamin D supplementation during lactation in the human is even scarcer than data with respect to vitamin D supplementation during pregnancy.
- As during pregnancy, an arbitrary AI has been set at 200 IU/d.

It is a well known fact that human milk is a poor source of vitamin D for the nursing infant.

This is an absolutely false and absurd statement.

Vitamin D and Lactation

- Can maternal vitamin D supplementation satisfy both maternal and infant requirements?

Prior Studies Involving Vitamin D Supplementation to Lactating Mothers

- Ala-Houhala et al (1986) supplemented lactating mothers with 1,000 – 2,000 IU/d vitamin D₃ for 15 weeks.
- Increases in circulating 25(OH)D levels were observed in both mother and infant with a more pronounced effect in the higher intake group.

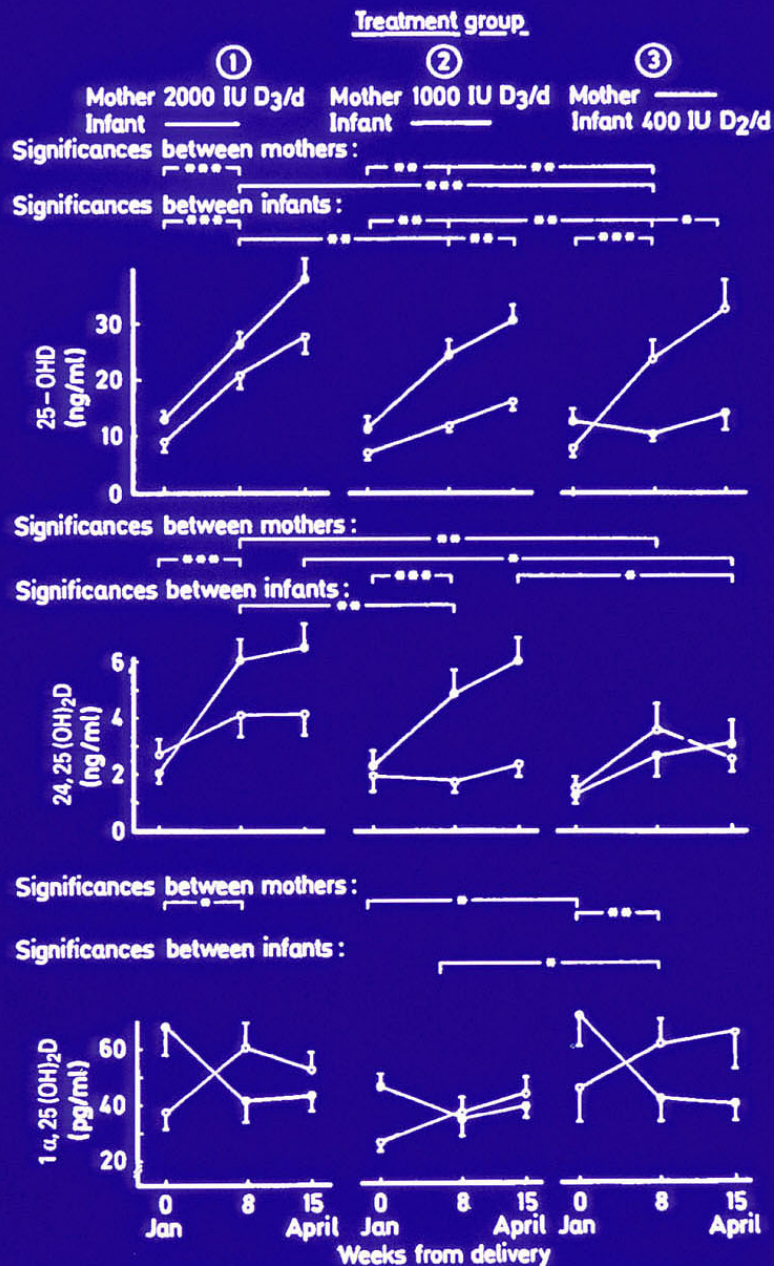
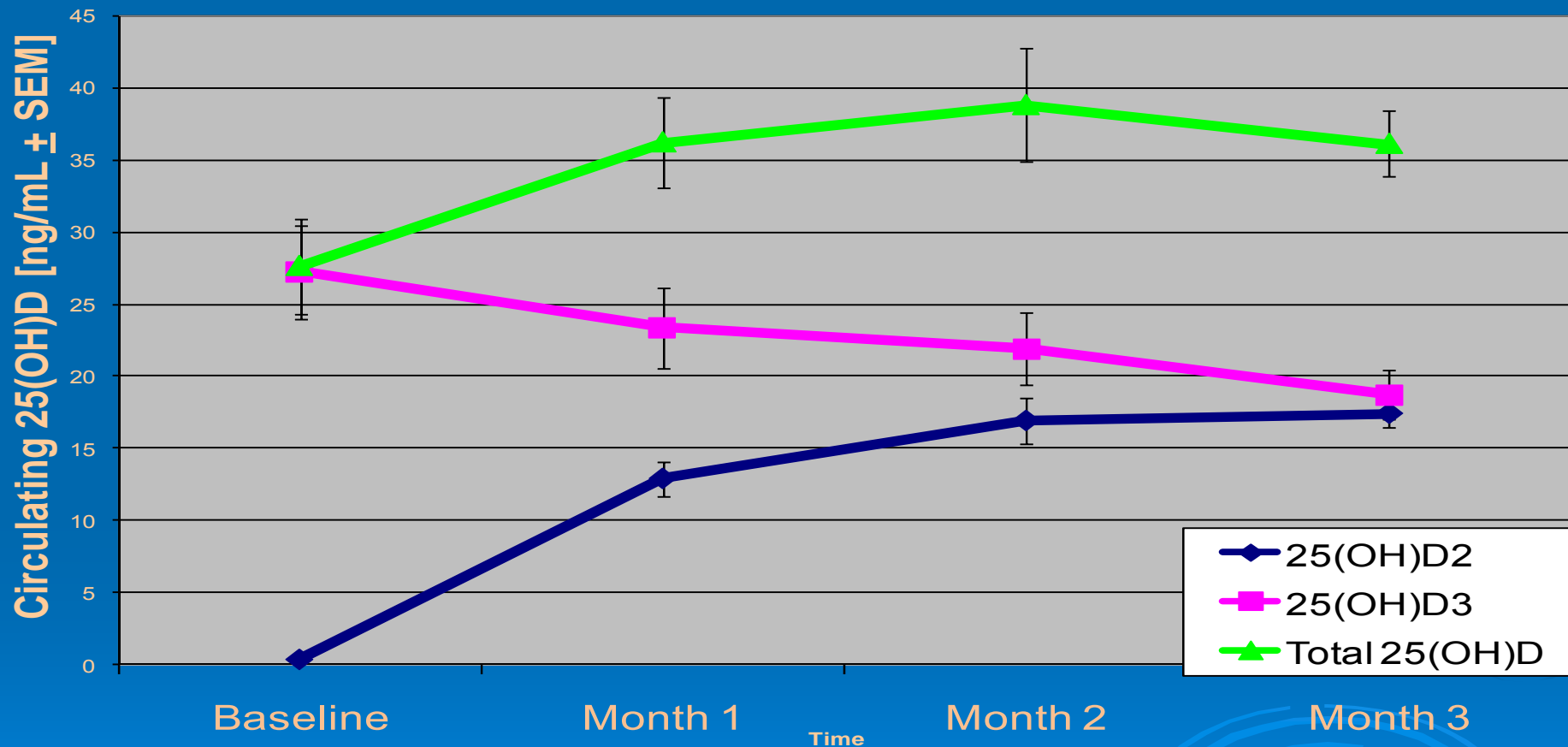


Fig. 1 Serum vitamin D metabolite concentrations (mean (SEM)) in winter of mothers (●) and infants (○) in different groups with vitamin D supplementation at delivery and 15 weeks later.

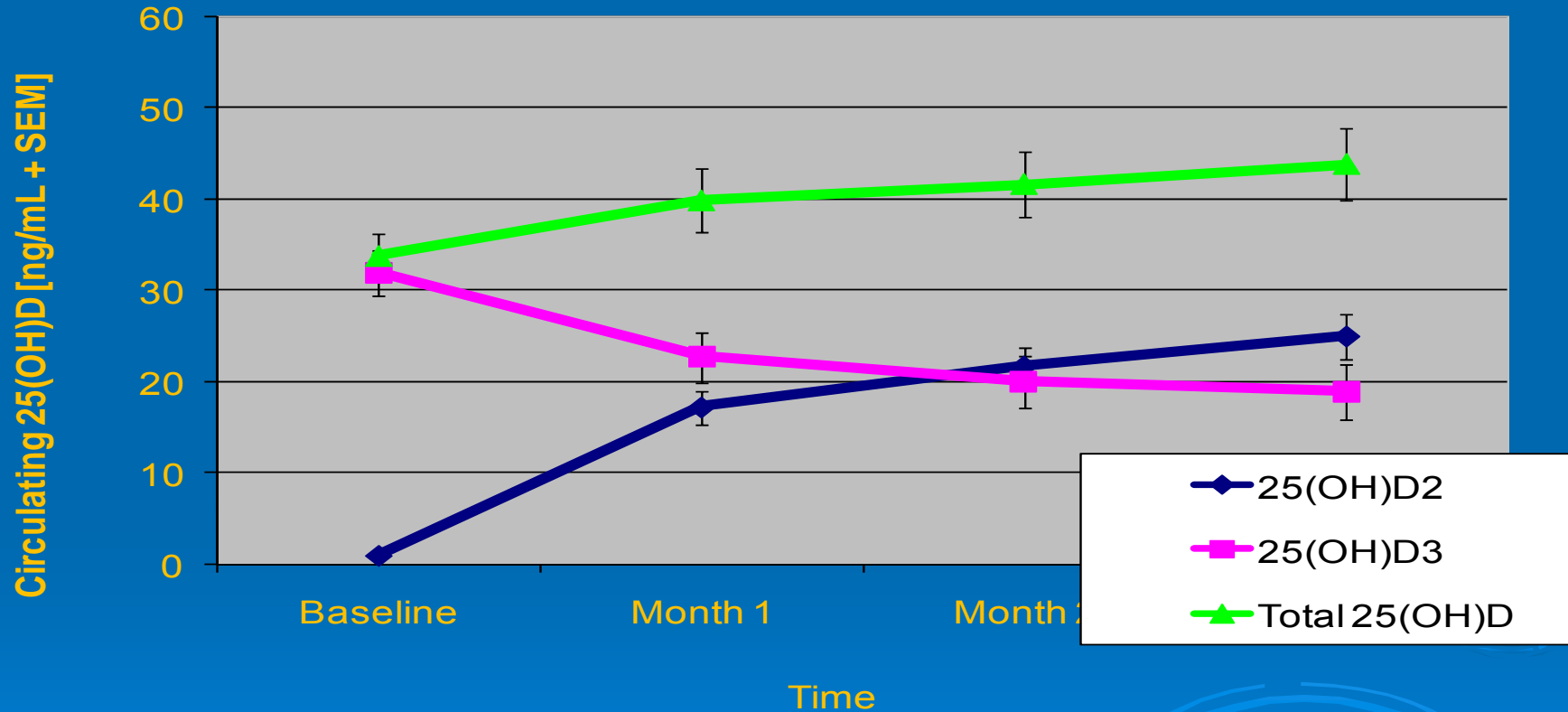
Prior Studies (con't)

- **Hollis and Wagner (2004) supplemented lactating mothers with 2,000 or 4,000 IU/d vitamin D₂ for three months.**
 - This study investigated what this level of supplementation would achieve with respect to increasing circulating levels of vitamin D and 25(OH)D in the mother and her nursing infant.
 - It was also of importance to see the effect of this supplementation level on milk antirachitic activity.
 - The results were somewhat disappointing.

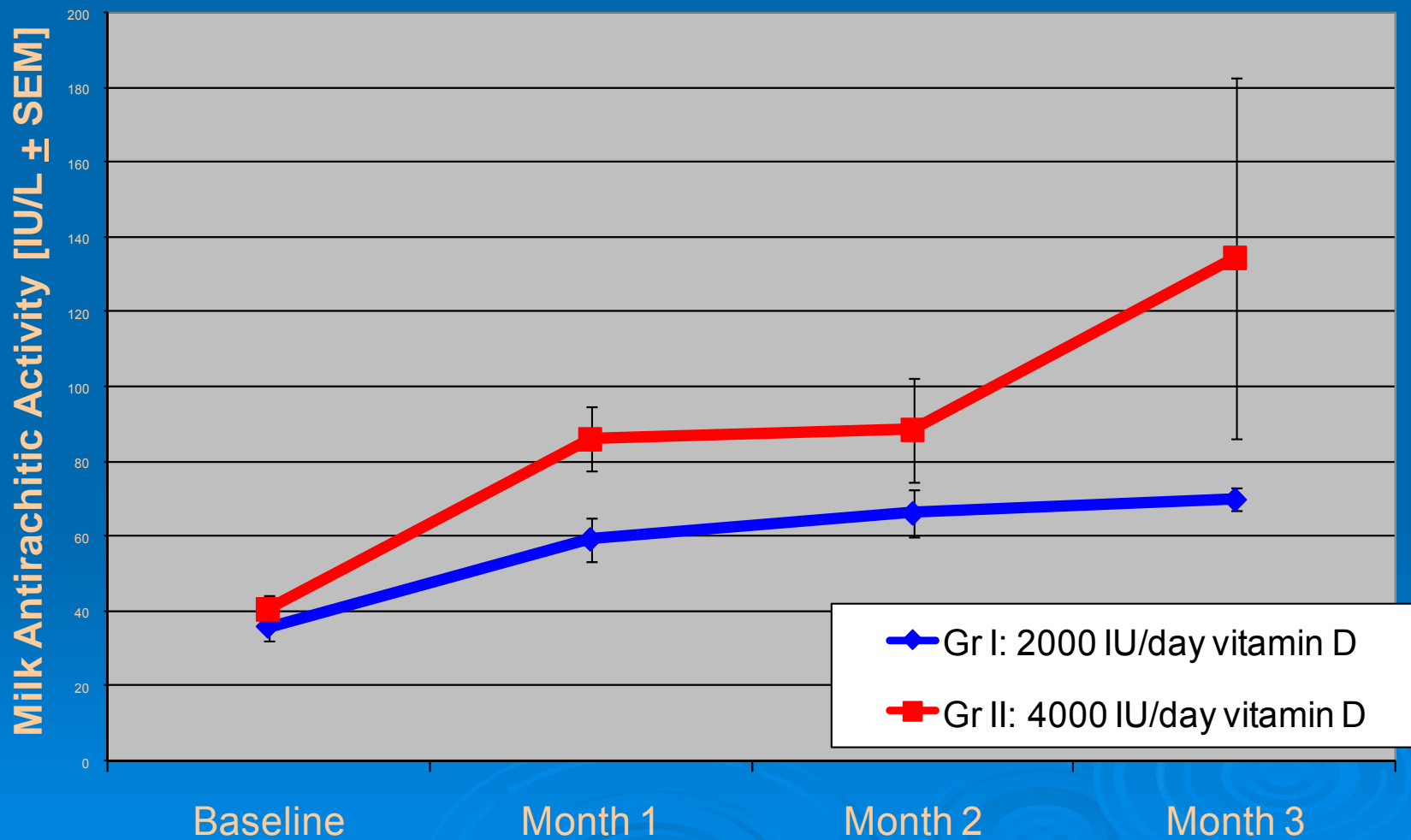
Longitudinal Assessment of Serum 25(OH)D in Lactating Women Receiving 1,600 IU/day Vitamin D₂ and 400 IU/day Vitamin D₃ (n=9)



Longitudinal Assessment of Serum 25(OH)D in Lactating Women Receiving 3,600 IU/day Vitamin D2 and 400 IU/day Vitamin D3 (n=9)



Longitudinal Assessment of Milk Antirachitic Activity as a Function of Supplementation Regimen in Lactating Women (n=18)



Prior Studies (con't)

- Wagner et al (2006) supplemented lactating women with either 400 or 6,400 IU/d vitamin D₃ for six months. Their nursing infants received either 300 IU/d vitamin D₃ or placebo.
 - The results were quite remarkable as demonstrated on the following slides.

Maternal Supplementation with 400 IU vitamin D₃/day & Infant Supplementation with 300 IU/day (n=6)

		Visit (months)						
		V ₀	V ₁	V ₂	V ₃	V ₄	V ₅	V ₆
Vitamin D ₃ (ng/mL ± SD)	Mother	2.4 ± 2.8	2.8 ± 1.5	3.5 ± 1.2	2.8 ± 1.9	3.7 ± 2.3	5.3 ± 3.5	12 ± 15
25(OH)D (ng/mL ± SD)	Mother	35 ± 10	35 ± 7	35 ± 4	30 ± 4	26 ± 9	35 ± 5	38 ± 8
	Baby	13 ± 8			33 ± 6			43 ± 7
Milk Activity (IU/L)		62 ± 17	71 ± 36	79 ± 33	54 ± 18	68 ± 36	70 ± 25	147 ± 138

Maternal Supplementation with 6,400 IU Vitamin D₃/day only (n=6)

		Visit (months)						
		V ₀	V ₁	V ₂	V ₃	V ₄	V ₅	V ₆
Vitamin D ₃ (ng/mL ± SD)	Mother	4.6 ± 3.9	32 ± 12	38 ± 9	39 ± 27	52 ± 15	44 ± 15	47 ± 19
25(OH)D (ng/mL ± SD)	Mother	36 ± 12	48 ± 12	50 ± 10	52 ± 13	51 ± 9	53 ± 10	57 ± 14
	Baby	14 ± 6			36 ± 8			46 ± 10
Milk Activity (IU/L)		90 ± 27	403 ± 173	419 ± 214	379 ± 202	597 ± 329	623 ± 408	782 ± 428

Summary

- The current AI (200 IU/d) for vitamin D during lactation is irrelevant to the mother and her nursing infant.
- The vitamin D requirement during lactation is likely the greatest for any physiological human condition and may exceed 6,000 IU/d.

**Establishing the Vitamin
D Requirement During
During Lactation**

NIH # R01 HD047511-01

Specific Aim

- Determine the efficacy, effectiveness and safety of maternal and infant vitamin D supplementation as a function of maternal vitamin D status, lactation status, ethnicity and latitude in the prevention of hypovitaminosis D.

Study Design

- 5-Yr Prospective, two-site, randomized, double-blind placebo-controlled vitamin D supplementation trial.
 - Lactating and non-lactating women stratified by ethnicity/race
 - African American (n=126)
 - Caucasian (n=126)
 - Hispanic (n=126)

Study Design (con't)

- Each ethnic/racial and lactating group randomized to one of three vitamin D₃ doses for six months.
 - 63 women in each subgroup
 - Oral vitamin D doses for mother/infant
 - 400 IU/d maternal; 400 IU/d infant (control)
 - 2,400 IU/d maternal; placebo infant
 - 6,400 IU/d maternal; placebo infant

Conclusion

- The current AI of 200 IU/d for vitamin D during pregnancy and lactation in humans is meaningless with respect to the true requirement which will be determined in the years to come.

What did you learn?

- **What form to measure?**
 - 25(OH)D
- **The desired amount?**
 - 40-100 ng/ml (100-250 nmol)
- **How to obtain this level?**
 - 1,000 – 6,000 IU/d vitamin D
- **Health consequences of too little?**
 - Dire

Thank you!

