VITAMIN D SUPPLEMENTATION DURING PREGNANCY AND IT'S EFFECT ON PREGNANCY OUTCOMES

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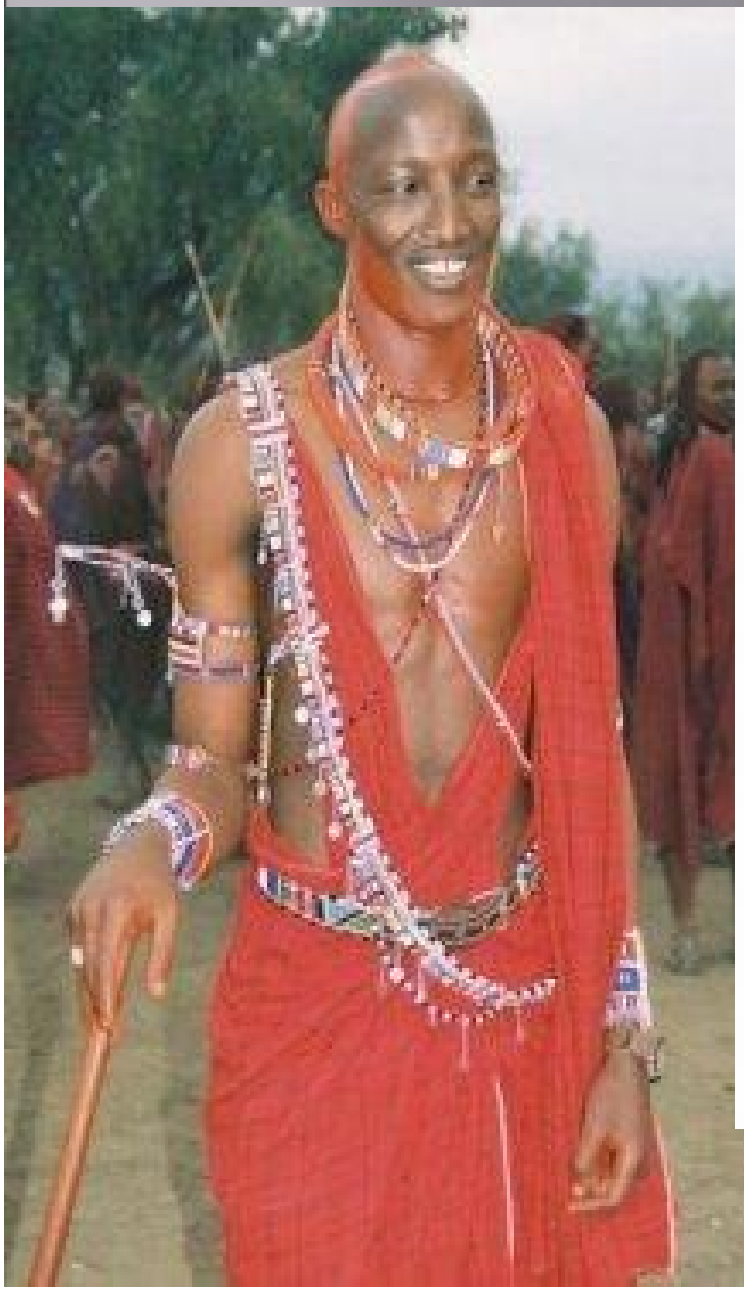
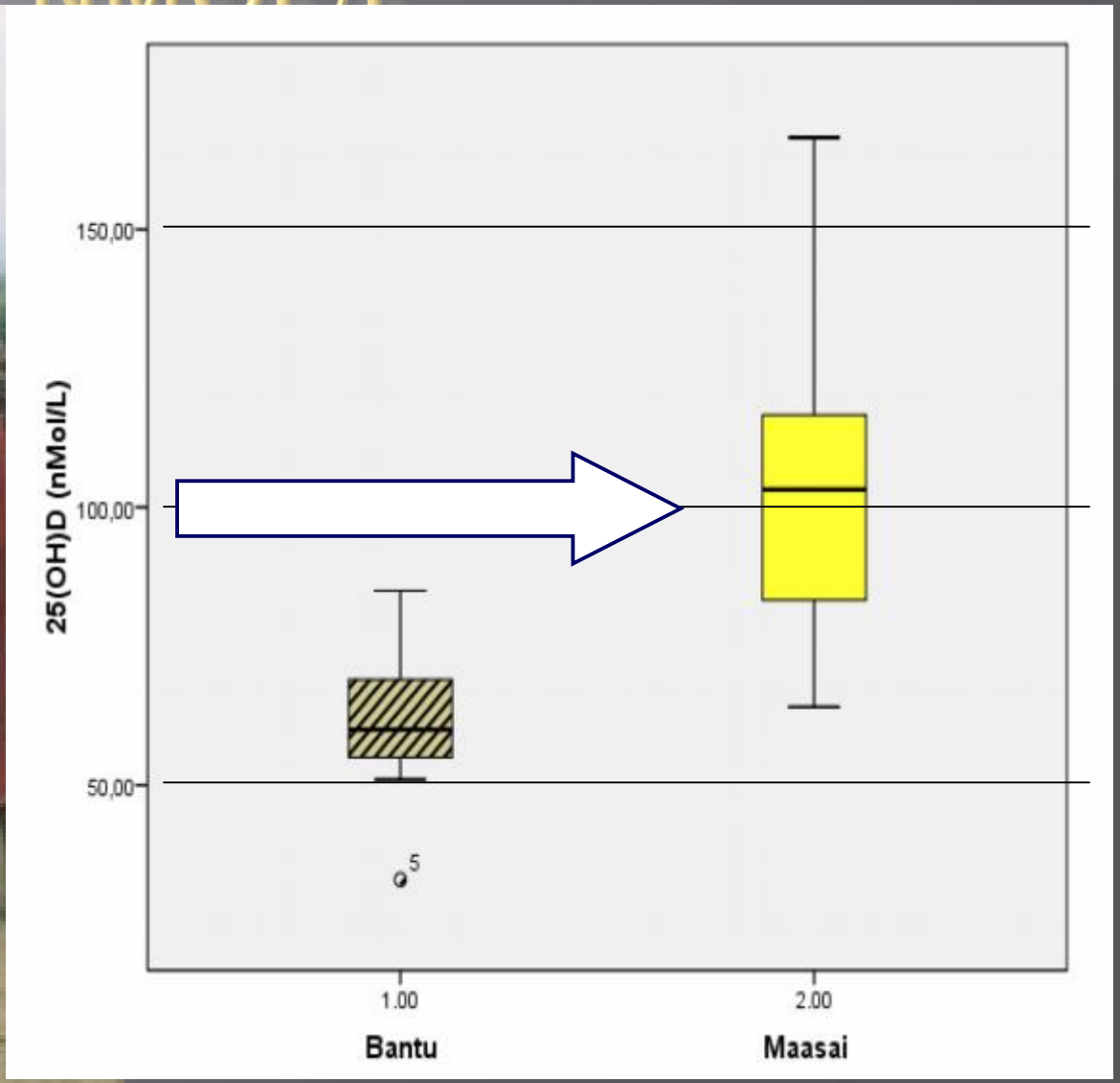
LEARNING OBJECTIVES

Upon completion of this educational activity, participants will be able to:

1. Recognize the risk factors for inadequate vitamin D status.
2. Identify the basic aspects of vitamin D metabolism.
3. Review recent literature linking inadequate vitamin D status and adverse pregnancy outcomes

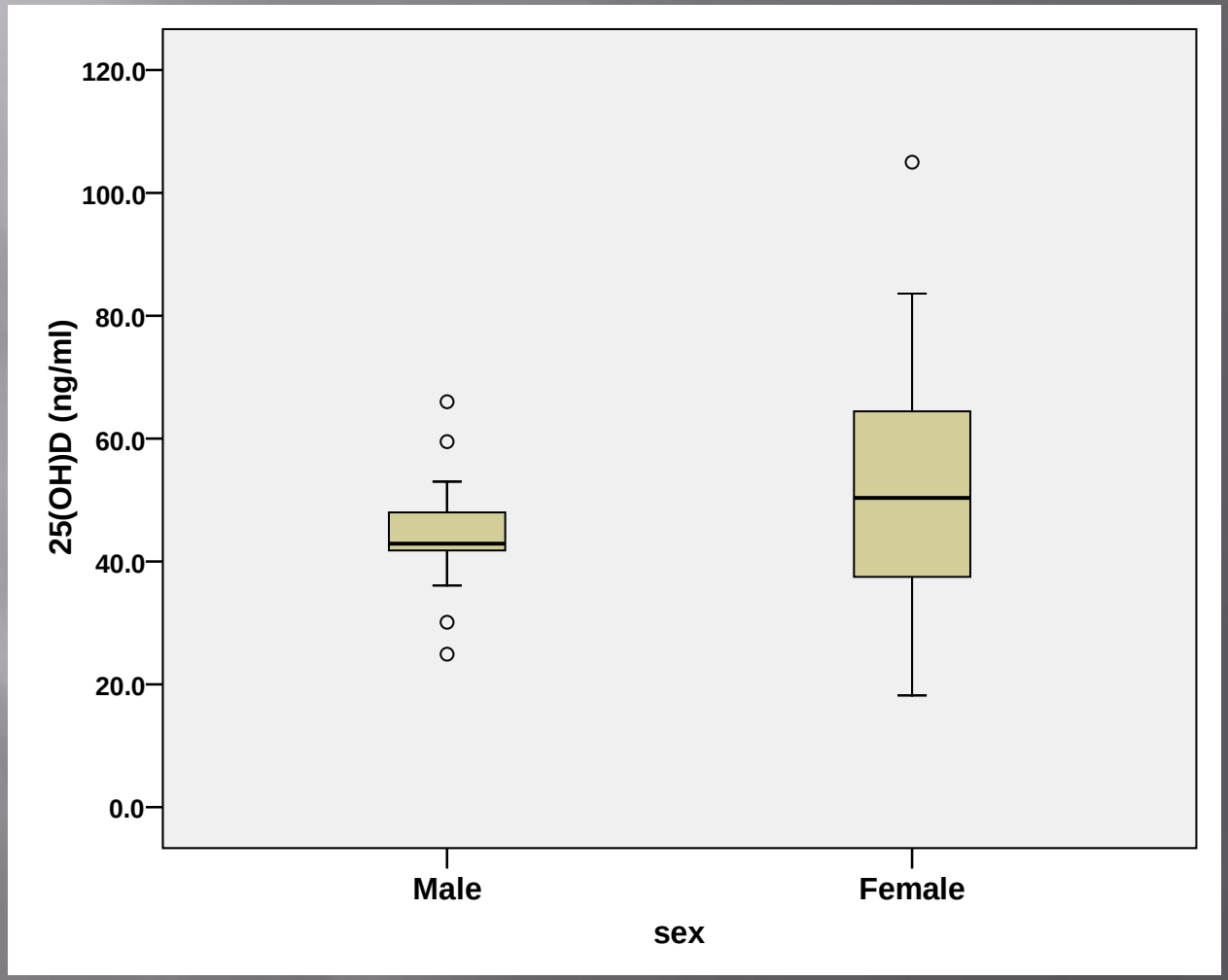
MAASAI MEDIAN 25(OH)D = 104 NMOL/L





Luxwolda and Muskiet , submitted manuscript

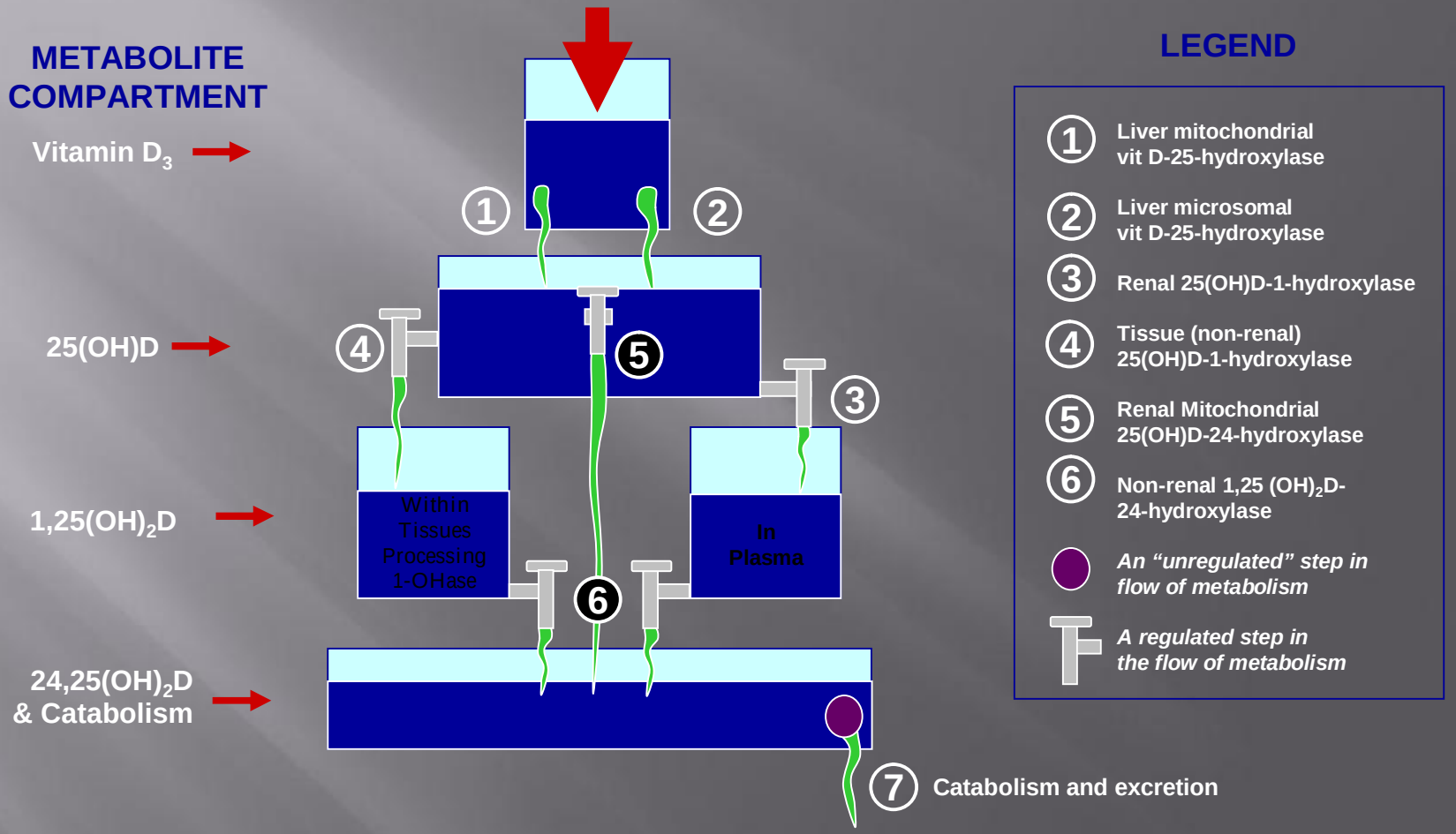
Laramie, WY Athletes: No Supplementation



The recent IOM recommendation states that a circulating level of 50 nmol 25(OH)D during pregnancy is desirable.

Metabolism of Vitamin D Under Conditions of Adequate Vitamin D Supply

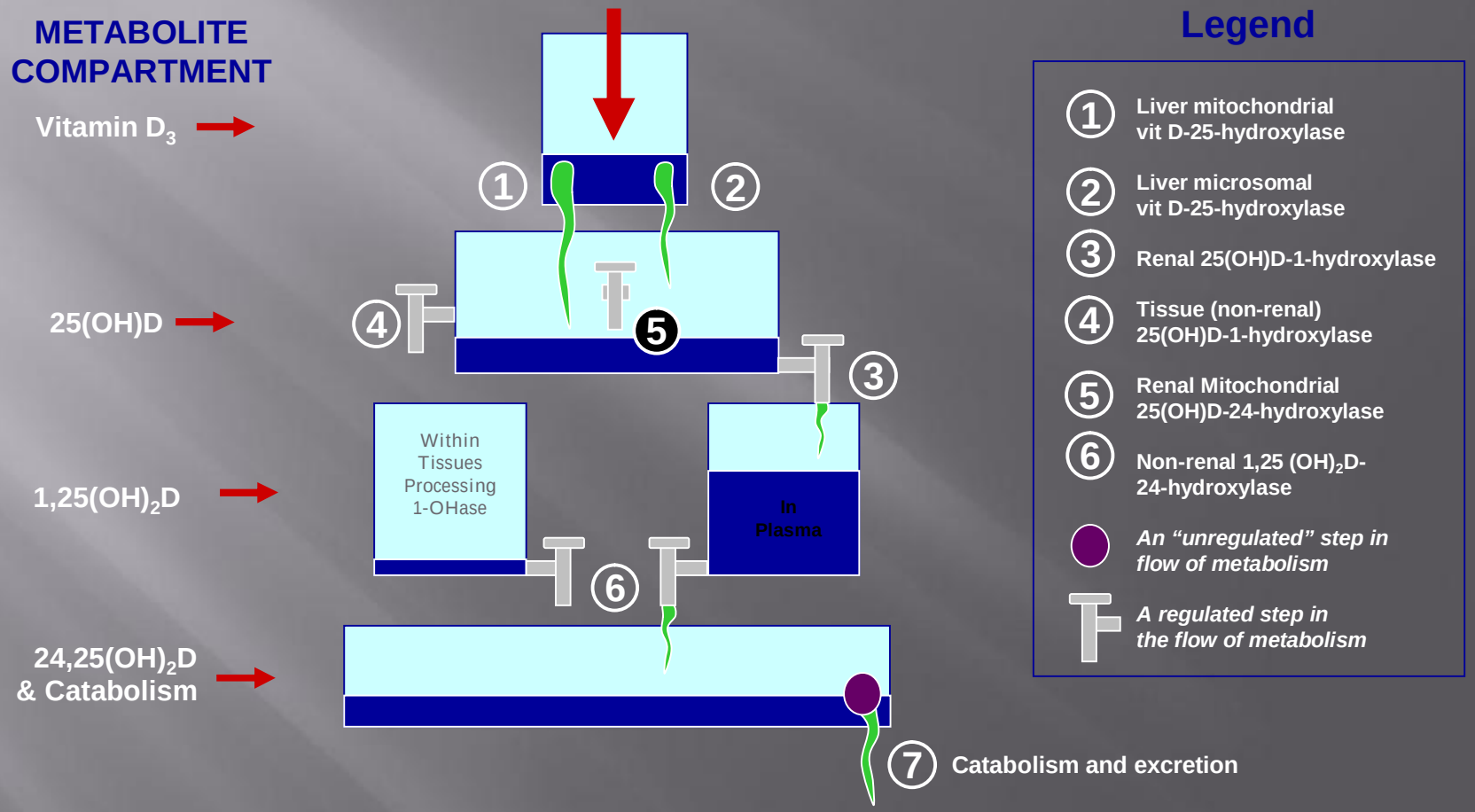
High/Normal Input of Cholecalciferol from diet or UVB



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



Legend

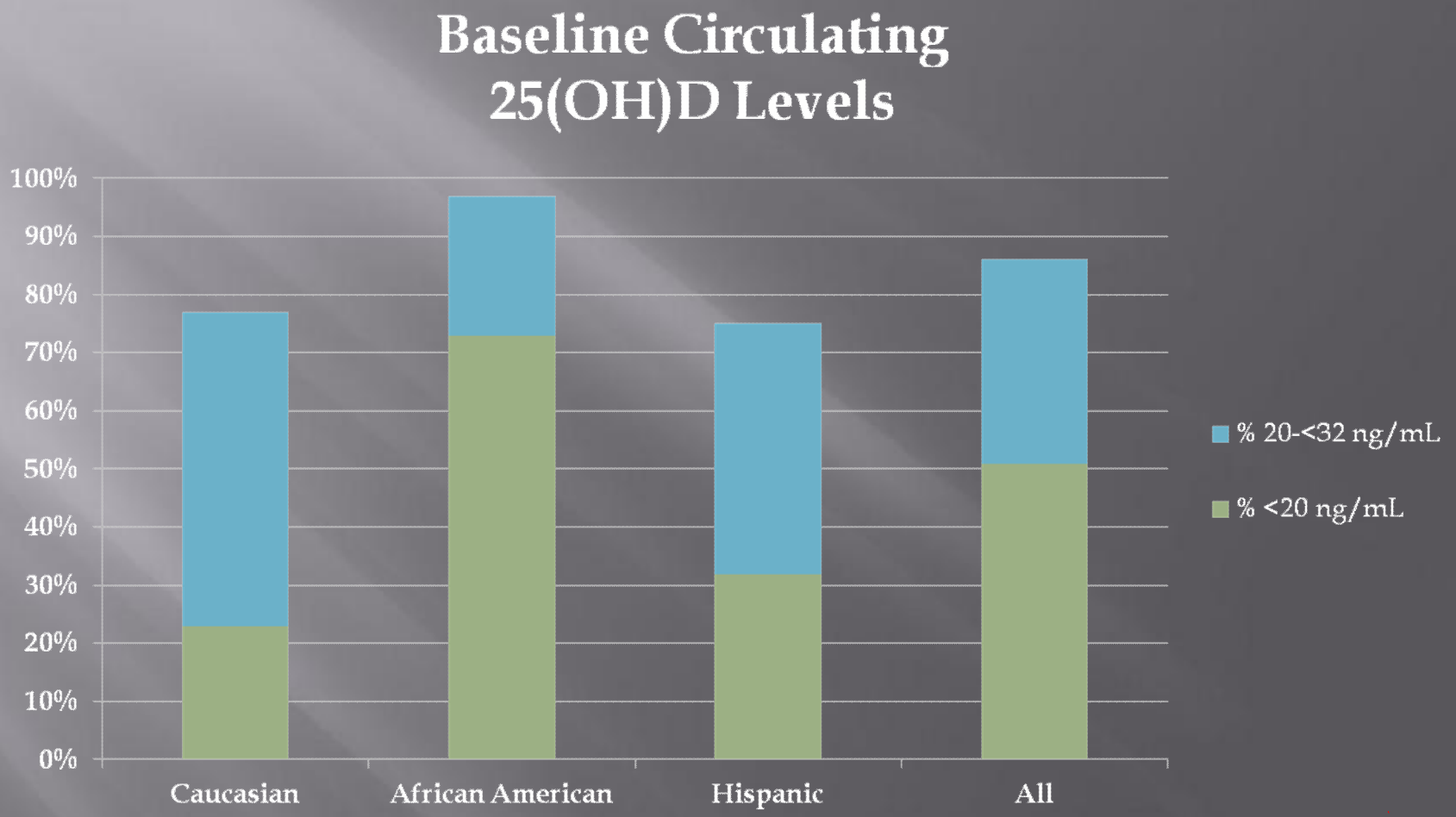
1. Liver mitochondrial vit D-25-hydroxylase
2. Liver microsomal vit D-25-hydroxylase
3. Renal 25(OH)D-1-hydroxylase
4. Tissue (non-renal) 25(OH)D-1-hydroxylase
5. Renal Mitochondrial 25(OH)D-24-hydroxylase
6. Non-renal $1,25(OH)_2D$-24-hydroxylase

An "unregulated" step in flow of metabolism

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.

Evidence of the Deficiency in Pregnant Women in a Sunny Climate, Latitude 32°N



Johnson D, et al. Am J Ob Gyn ; 2010
Hamilton S, et al. Int J Endocrinol; 2011

Public Health Issue

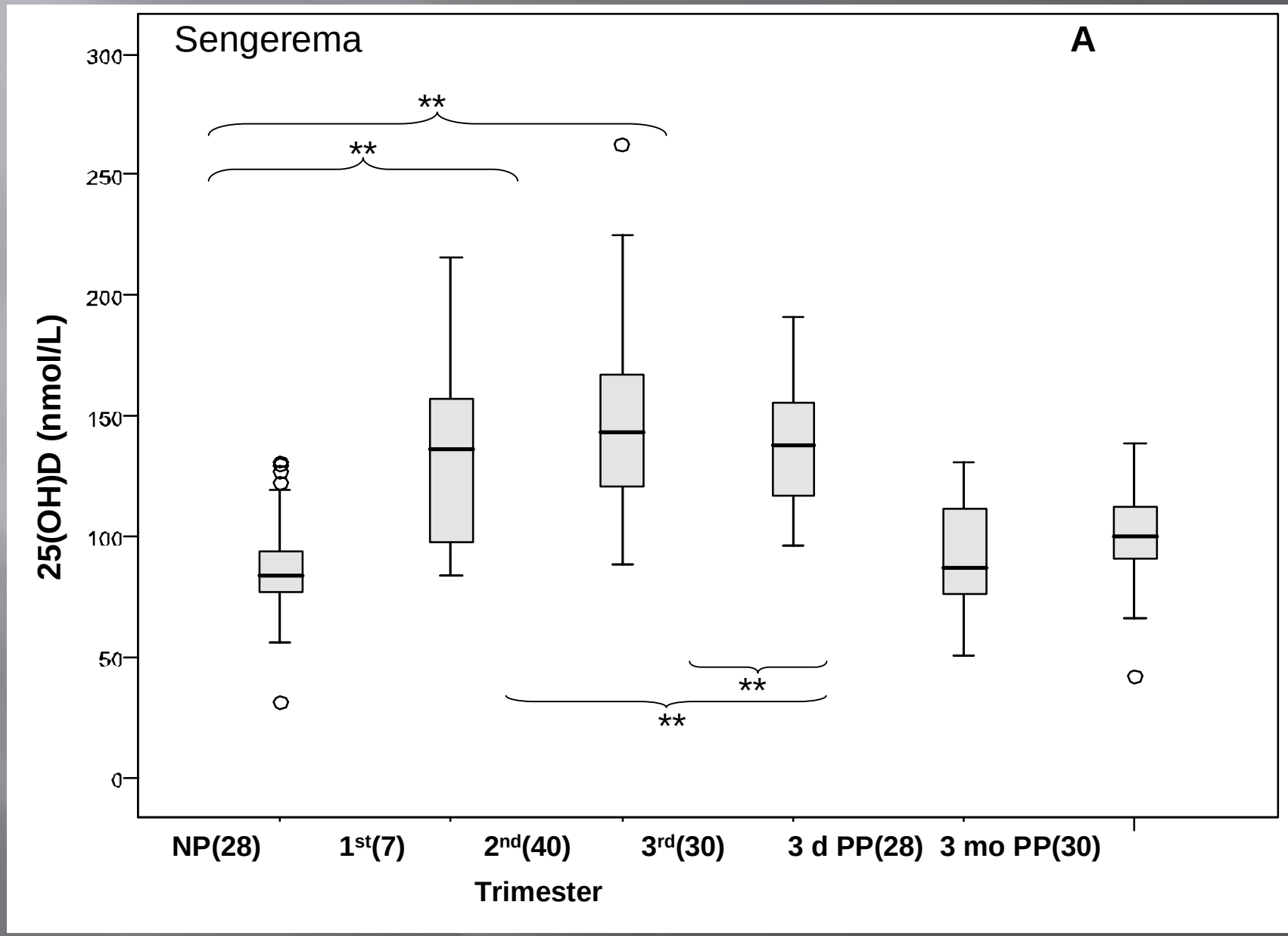
- Evident that vitamin D deficiency during pregnancy is a serious public health issue that affects both mother and fetus.
- Need to establish the vitamin D requirements of the pregnant woman seen as vital in preventing vitamin D deficiency.
- Yet, the recent Institute of Medicine made only a slight increase to vitamin D's RDA—from 400 IU to 600 IU / day.
  - What is the truth?

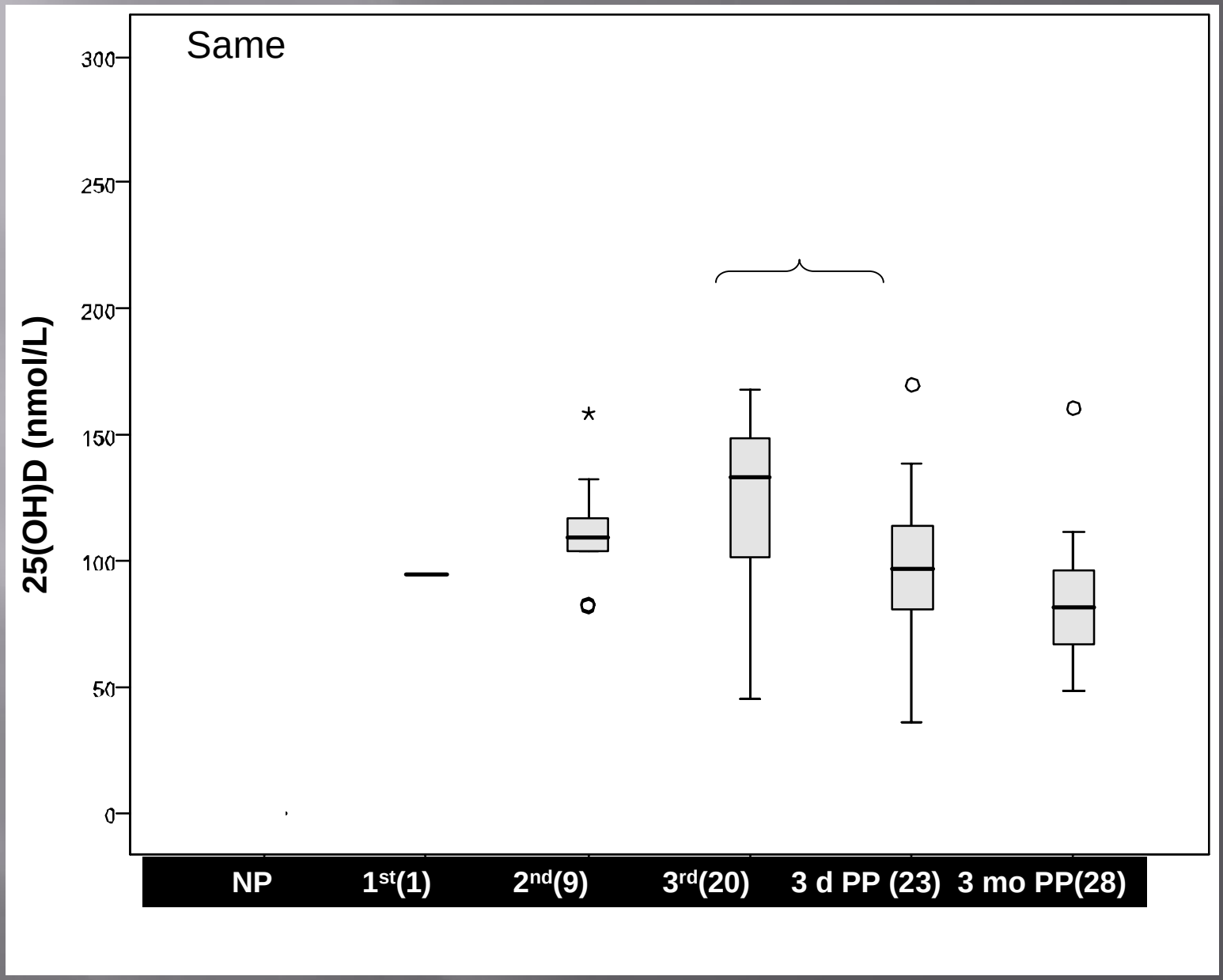
Table 1. Summary of RCT Vitamin D Supplementation Studies During Pregnancy

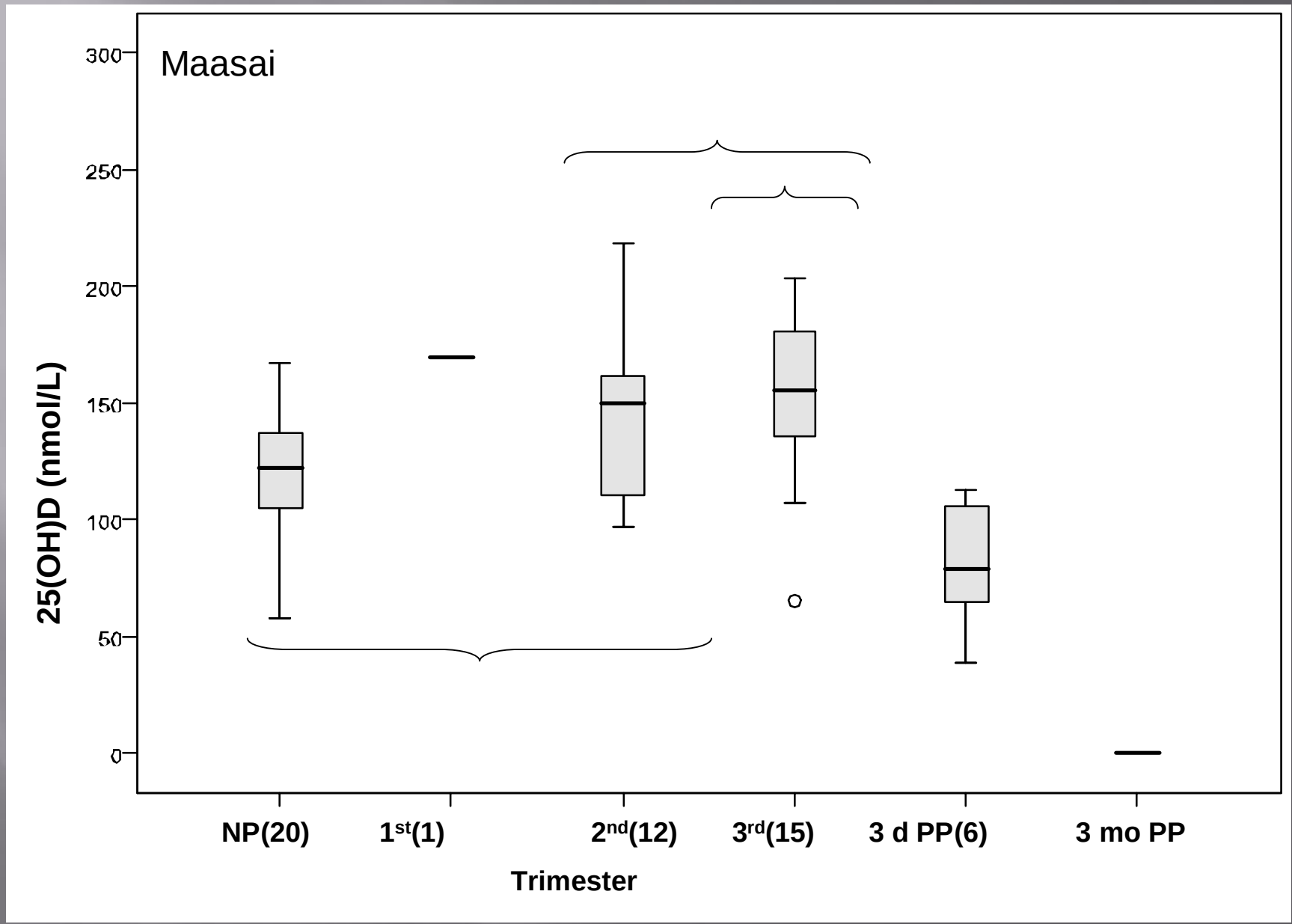
Reference	*Number of subjects*	*Vitamin D dose (IU/d)*	*Therapy duration (mo)*	*Initial 25(OH)D (nmol/L)*	Endpoint 25(OH)D (nmol/L)
Brooke et al.[10]*	67 Control 59 Supplemented	0 1000 D_2	8.0 3	16.3 20.0	 168.0
Cockburn et al.[12]	82 Control 82 Supplemented	0 400D_2	13.0 4	 32.5 39.0	 42.8
Brooke et al.[10]	67 Control 59 Supplemented	0 1000 D_2	? 3	 ? ?	 ?
Maxwell et al.[13]	67 Control 59 Supplemented	0 1000 D_2	8.0 3	? 20.0	 ?
Marya et al. [35]	75 Control 25 Supplemented	0 1200 D_2	? 3	 ? ?	 ?
Delvin et al. [36]	15 Control 15 Supplemented	0 1000 D_3	? 3	17.5 (cord) ?	 45.0 (cord)
Mallet et al.[37]	27 Control 21 Supplemented	0 1000 D_2	? 3	9.5 ?	 25.3
Ho Hollis et al.[1] †	111 Supplemented 122 Supplemented 117 Supplemented	400 D_3 2,000 D_3 4,000 D_3	6 6 6	61.5 58.3 58.3	79.0 98.3 111.0

* It is very likely that the wrong dose of supplementation was given or the assay for 25(OH)D was invalid. The response observed is one that would be expected after supplementation with 10,000 IU/d vitamin D_3 for 3 mo. [10]

†It is important to note that the earlier studies with the exception of Hollis et al. [1] were conducted with the control group receiving 0 IU vitamin D/day. Since the standard of care for the past three decades in the U.S. is to give pregnant women 400 IU vitamin D/day included in the prenatal vitamin, it would be unethical to conduct a vitamin D supplementation trial involving pregnant women in the U.S. today with 0 IU vitamin D/day.

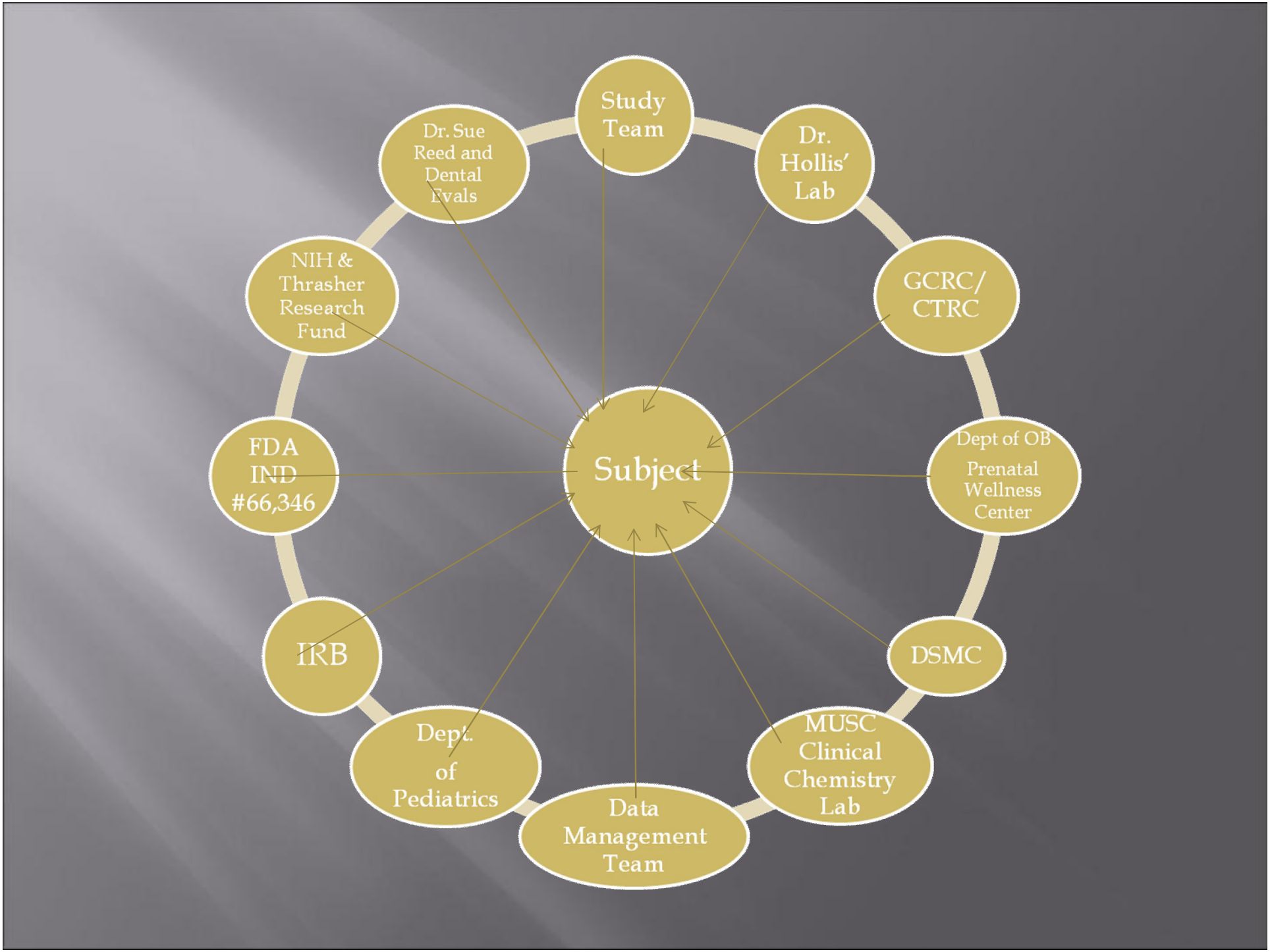

Sengerema
A
300
250
200
150
100
50
0
25(OH)D (nmol/L)
**
**
**
**
NP(28)
1st(7)
2nd(40)
3rd(30)
3 d PP(28)
3 mo PP(30)
Trimester


Same
25(OH)D (nmol/L)
300
250
200
150
100
50
0
NP
1st(1)
2nd(9)
3rd(20)
3 d PP (23)
3 mo PP(28)


Maasai
300
250
200
150
100
50
0
25(OH)D (nmol/L)
NP(20)
1st(1)
2nd(12)
3rd(15)
3 d PP(6)
3 mo PP
Trimester

Methods and Study Design

- Study Design: randomized control, double-blind placebo study of vitamin D supplementation.
- Women <16 weeks' singleton gestation eligible for participation in the study; those with any underlying diabetes or hypertension not eligible to participate
- Baseline 25(OH)D levels measured
  - Women with levels ≤40 ng / mL randomized to 400, 2000 or 4000 IU vitamin D_3 / day) with further stratification by race
  - Women with baseline 25(OH)D levels >40 ng / mL were randomized to either 400 or 2000 IU vitamin D_3 / day
  - Women with baseline 25(OH)D level >60 were given 400 IU vitamin D_3 / day
- Women evaluated for safety, efficacy and effectiveness with monthly 25(OH)D; $1,25(OH)_2D$; serum Ca, Cr, phosphorus; and urinary Ca / Cr levels
- Investigators & health team blinded to treatment group
- IND required by FDA: Br. Bruce Hollis obtained in May 2002—first ever awarded to an investigator for a "vitamin" / hormone


Study Team
Dr. Hollis' Lab
GCRC/ CTRC
Dept of OB Prenatal Wellness Center
DSMC
MUSC Clinical Chemistry Lab
Data Management Team
Dept. of Pediatrics
IRB
FDA IND #66,346
NIH & Thrasher Research Fund
Dr. Sue Reed and Dental Evals
Subject

Outcome Measures

- Analyzed by Intent-to-Treat (by treatment group) and by Efficacy (25-OH-D achieved at various time-points during pregnancy and at delivery
- Primary Outcome Variable:
  - Maternal and neonatal total circulating 25(OH)D at delivery
- Sample size calculation to reject the null hypothesis that high vitamin D supplementation (2000 or 4000 IU groups) would not significantly improve maternal and neonatal vitamin D status:
  - To detect a statistically significant increase in 25(OH)D by 10 ng / mL between any two groups: minimum of 32 patients per group at 80% power, alpha = 0.05, two tailed test for the primary analysis.
- Secondary Outcome Measures:
  - $1,25(OH)_2D$
  - PTH
  - Maternal and neonatal health outcome measures
    - with the null hypothesis that high dose maternal vitamin D supplementation groups compared to control would not differ in adverse events during pregnancy

Results

- Of the 494 women who enrolled in the study, 350 women continued until delivery:
  - 98 African American
  - 137 Hispanic
  - 115 Caucasian women
- There were:
  - 111 controls
  - 122 in 2000 IU
  - 117 in 4000 IU groups
- No differences in baseline 25(OH)D by dose group:
  - 24.6 ng / mL (61.5 nmoL / L) in Control (400 IU group)
  - 23.3 ng / mL (58.3 nmoL / L) in 2000 IU group
  - 23.5 ng / mL (58.8 nmoL / L) in 4000 IU group

Table 1. Sociodemographic and Maternal Clinical Characteristics at Study Enrollment by Vitamin D Supplementation Group

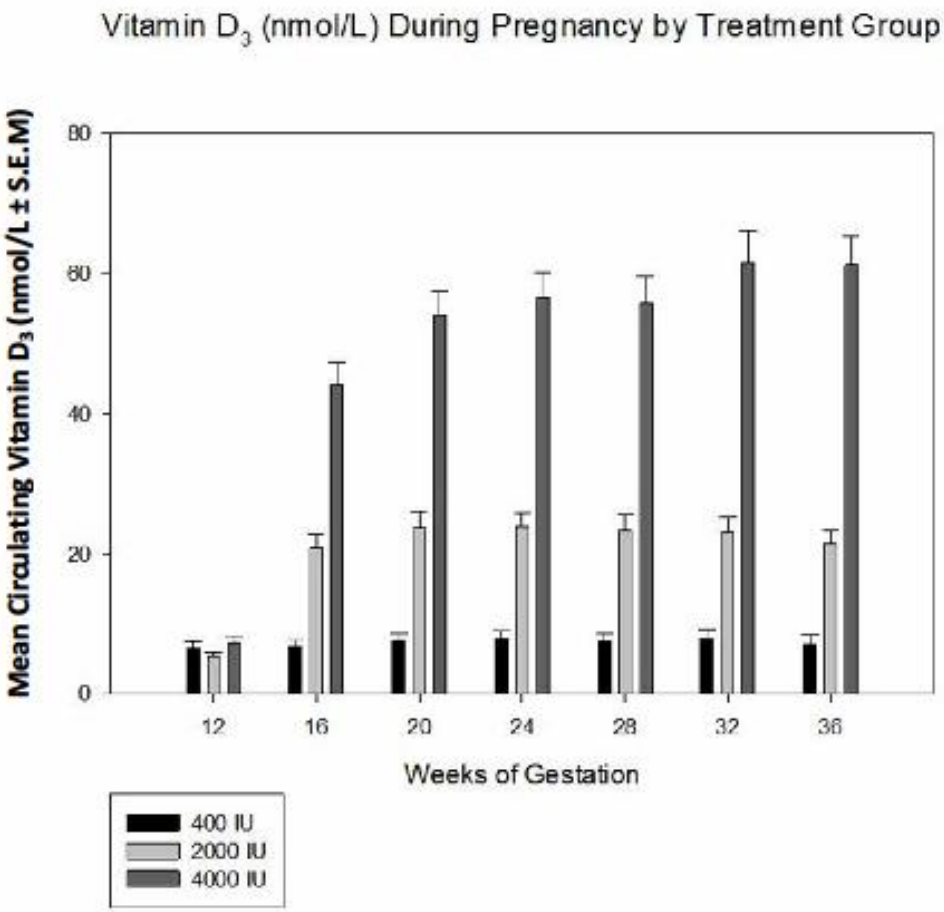
Characteristic	400 IU Group N=111	2000 IU Group N=122	4000 IU Group N=117	p-value
African American Hispanic Caucasian	28 (25.2) 45 (40.5) 38 (34.2)	37 (30.3) 48 (39.3) 37 (30.3)	33 (28.2) 44 (37.6) 40 (34.2)	 0.9
Maternal Age (Mean ± SD) (Range)	26.9 ±5.7 15 - 41	27.4 ±5.7 17 - 41	26.6 ±5.4 17 - 44	0.6
Gestational Age at Enrollment (Mean ± SD) (Range)	 12.5 ±1.9 7.1 – 18.4	 12.6 ±1.6 8.4 – 17.6	 12.4 ±2.0 6.4 – 21.4	0.8
Maternal Gravidity (Median) (Range)	2 1 - 8	2 1 - 7	2 1 -9	0.08
Maternal Parity (Median) (Range)	2 0-5	2 0-7	1 0 - 9	0.052
Education: N (%) < HS Education HS graduate College or more	 18 (17.3) 17 (16.4) 69 (66.4)	 23 (19.7) 24 (20.5) 70 (59.8)	 13 (11.6) 22 (19.6) 77 (68.8)	0.4
Employed at Entrance into Study N (%)	61 (55.0)	67 (54.9)	65 (55.6)	0.9
Insurance: N (%) Medicaid/None Commercial	 62 (55.9) 49 (44.1)	 85 (69.7) 37 (30.3)	 69 (59.0) 48 (41.0)	0.07

Subjective Health Rating Scale from 1 (poor) -10 (excellent) (Median) (Range)	9 5-10	10 5-10	10 1-10	0.4
Planned Pregnancy, N (%)	59 (54.6)	61 (50.4)	59 (50.4)	0.8
BMI: N (%) ≤30 > 30	 78 (70.3) 33 (29.7)	 87 (71.3) 35 (28.7)	 89 (76.1) 28 (23.9)	0.6
Season at study entry: N (%) April - September October - March	 54 (48.7) 57 (51.4)	 60 (49.2) 62 (50.8)	 56 (47.9) 61 (52.1)	0.9
Vitamin D Intake - (Mean ± SD) (Range)	181.6 ± 108.4 21.4 – 470.6	195.8 ± 135.0 8.2 - 693.8	204 .2± 148.2 5.3 – 737.3	0.6
Calcium Intake - (Mean ± SD) (Range)	1063.6 ± 539.6 252.9 – 2888.1	993.9 ± 514.0 285.4 – 2754.1	1073.6 ± 491.9 275.6 –2925.9	0.6
Kcal Intake - (Mean ± SD) (Range)	2148.3 ± 778.6 977.3- 4668.2	2059.4 ± 803 993.4 – 4793.4	2212.9 ± 920.8 929.3 – 5516	0.5

Mean 25(OH)D

- Intent-to-Treat Analyses:
  - Varied by treatment group at delivery
  - Varied as chronic status measured by area under the curve
  - 1-month before delivery
    - significantly different between:
      - Control *vs.* 2000 IU group ($p<0.0001$)
      - Control vs. 4000 IU group ($p<0.0001$)
      - 2000 vs. 4000 IU group ($p<0.0001$)

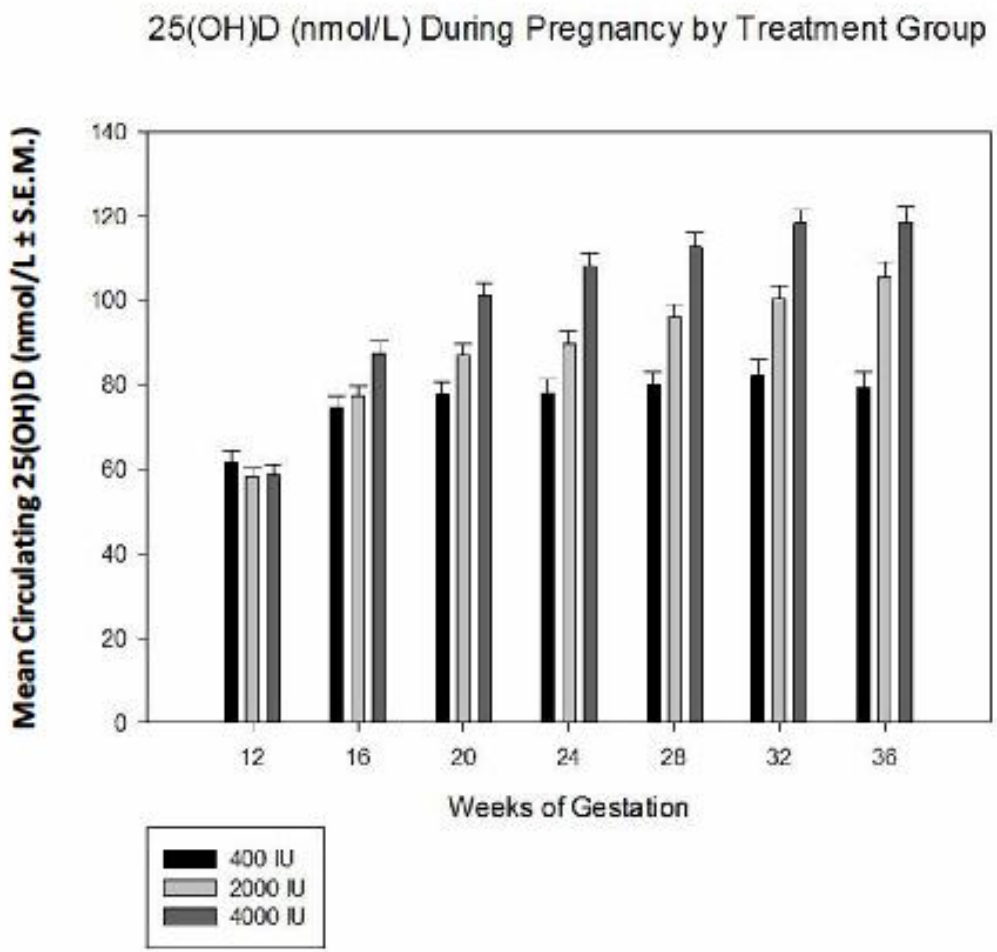
Figure 2a.



Statistical difference (p-value) in mean Vitamin D_3 between treatment groups at each week of gestation

Treatment Group	12	16	20	24	28	32	36
400 IU vs. 2000 IU	0.3	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
400 IU vs. 4000 IU	0.6	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
2000 IU v 4000 IU	0.1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

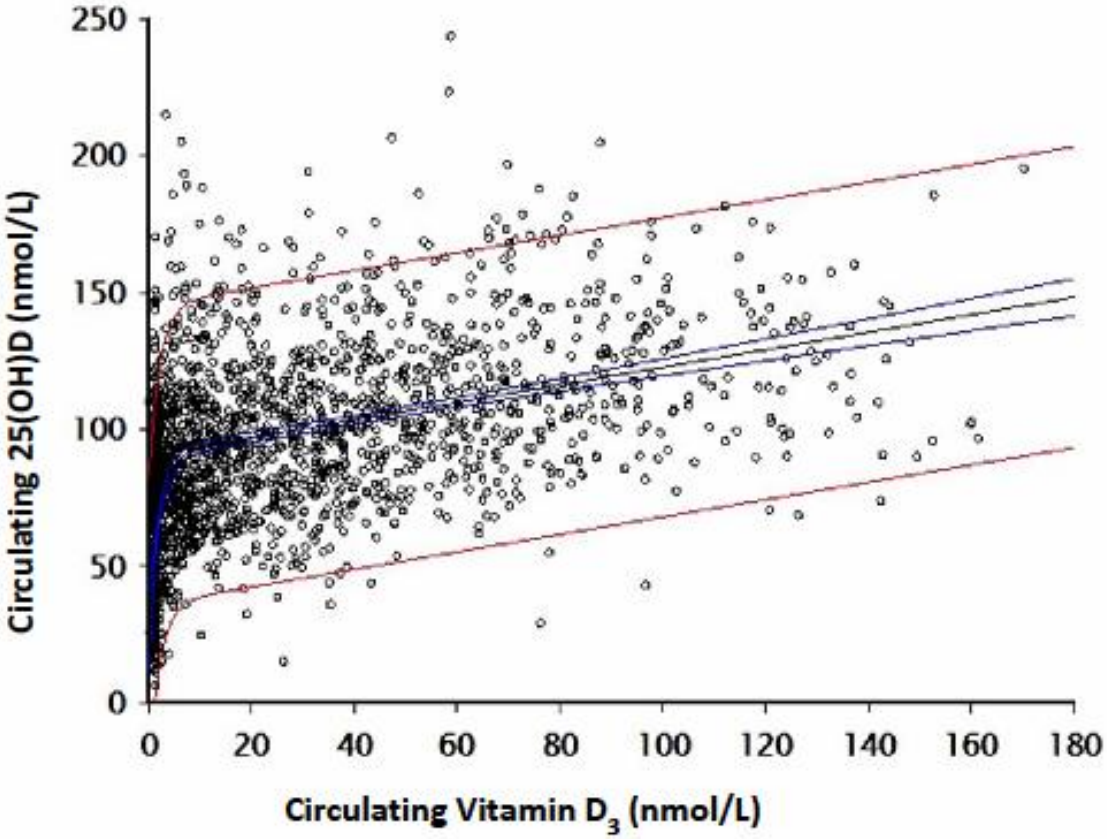
Figure 2b.



Statistical difference (p-value) in mean 25(OH)D between treatment groups at each week

Treatment Group	12	16	20	24	28	32	36
400 IU vs. 2000 IU	0.3	0.4	0.02	0.007	0.0001	0.0002	<0.0001
400 IU vs. 4000 IU	0.4	0.002	<.0001	<0.0001	<0.0001	<0.0001	<0.0001
2000 IU v 4000 IU	0.9	0.008	0.009	<0.0001	0.0003	<0.0001	0.009

Figure 3a.



25(OH)D = 26.4 + 64.2* (1—exp (-0.48*x)) + 0.32*x
R^2=0.37; p<0.0001

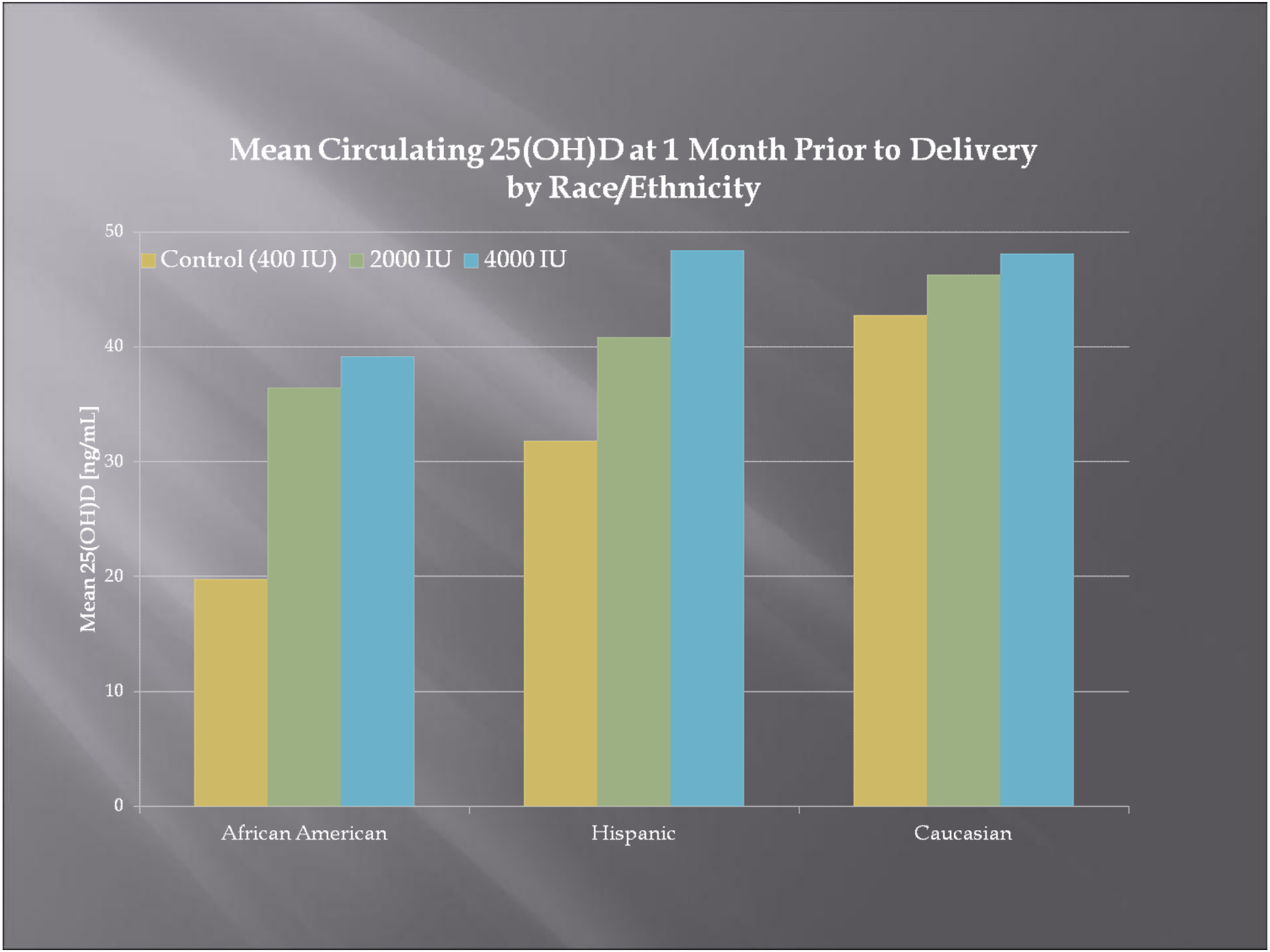
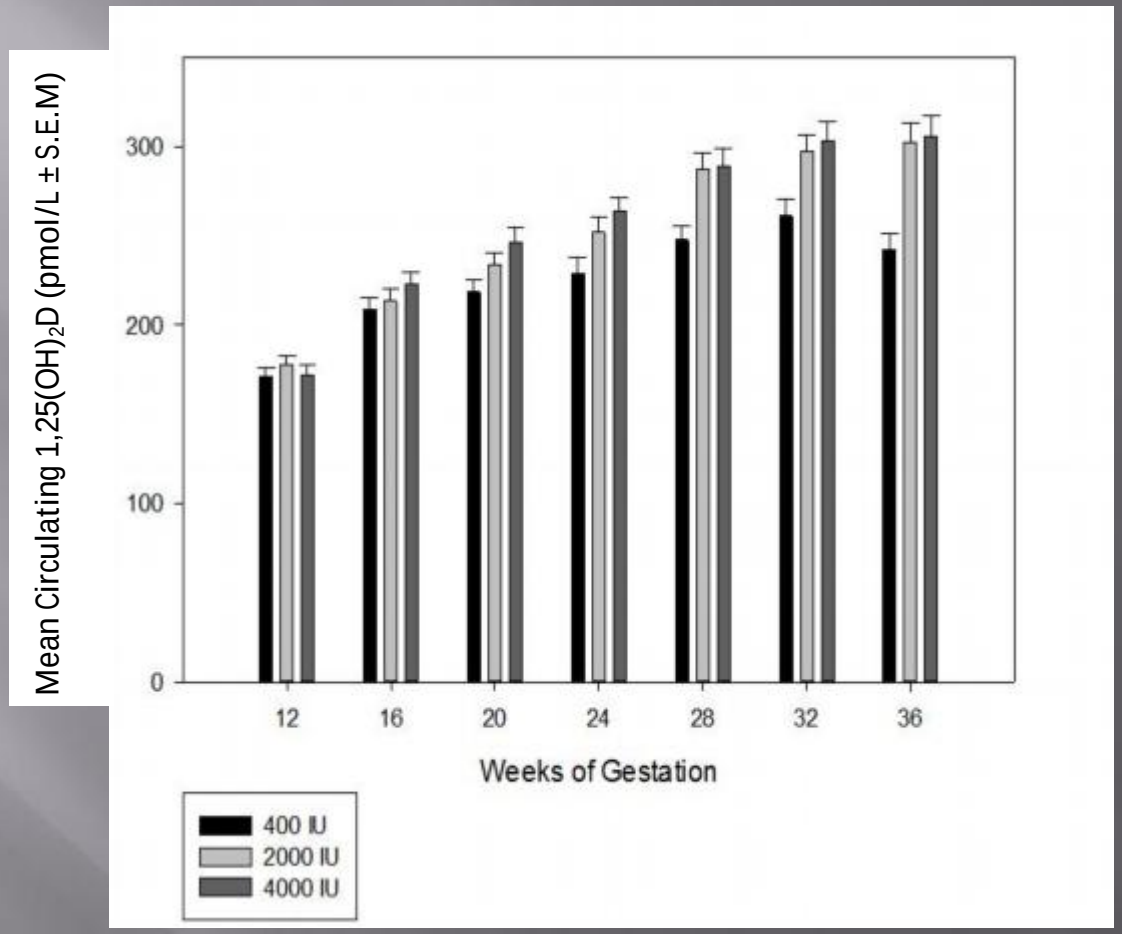

Mean Circulating 25(OH)D at 1 Month Prior to Delivery by Race/Ethnicity
Control (400 IU)
2000 IU
4000 IU
Mean 25(OH)D [ng/mL]
50
40
30
20
10
0
African American
Hispanic
Caucasian

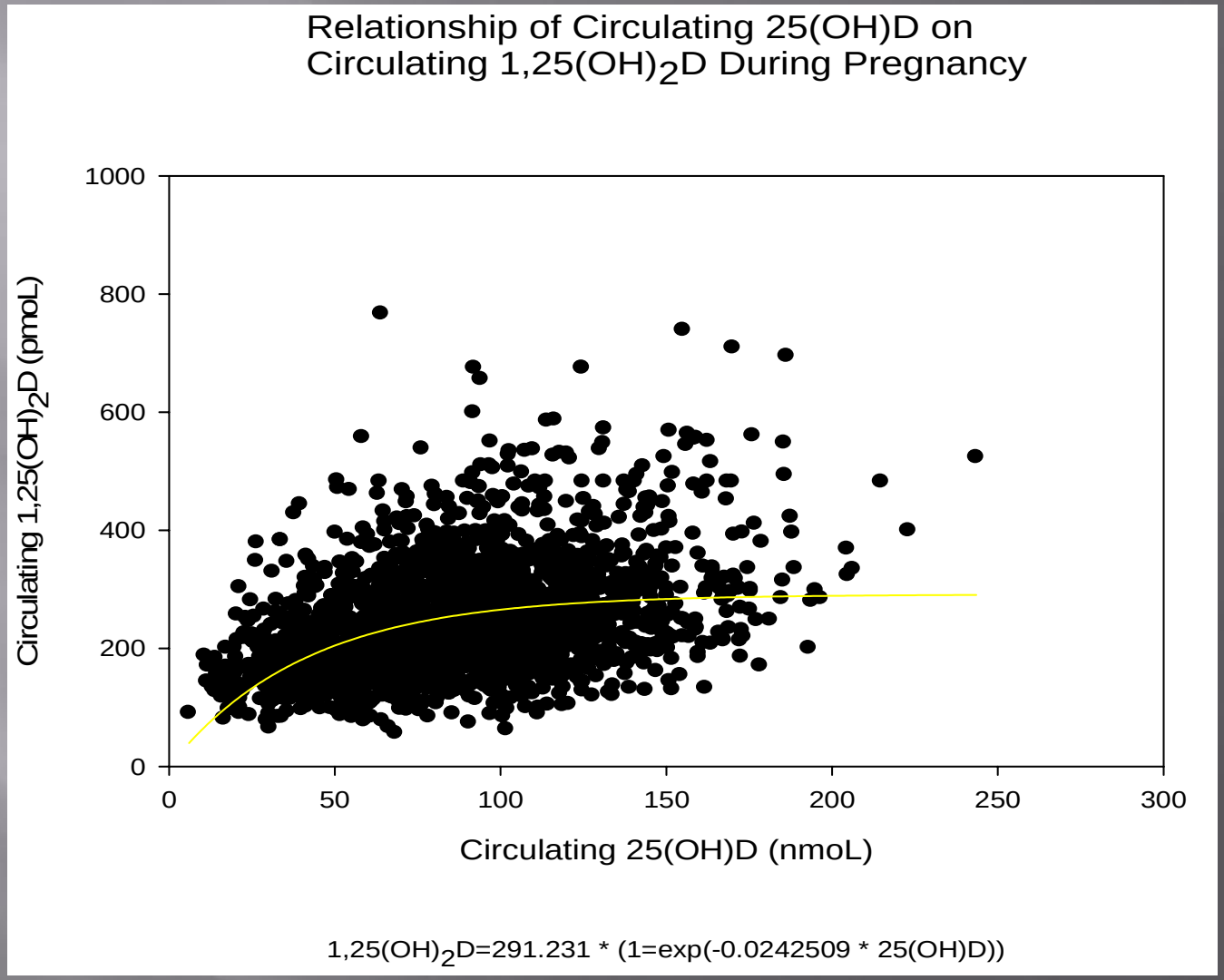
Figure 2c.

Circulating $1,25(OH)_2D$ (pmol/L) during Pregnancy by Treatment Group



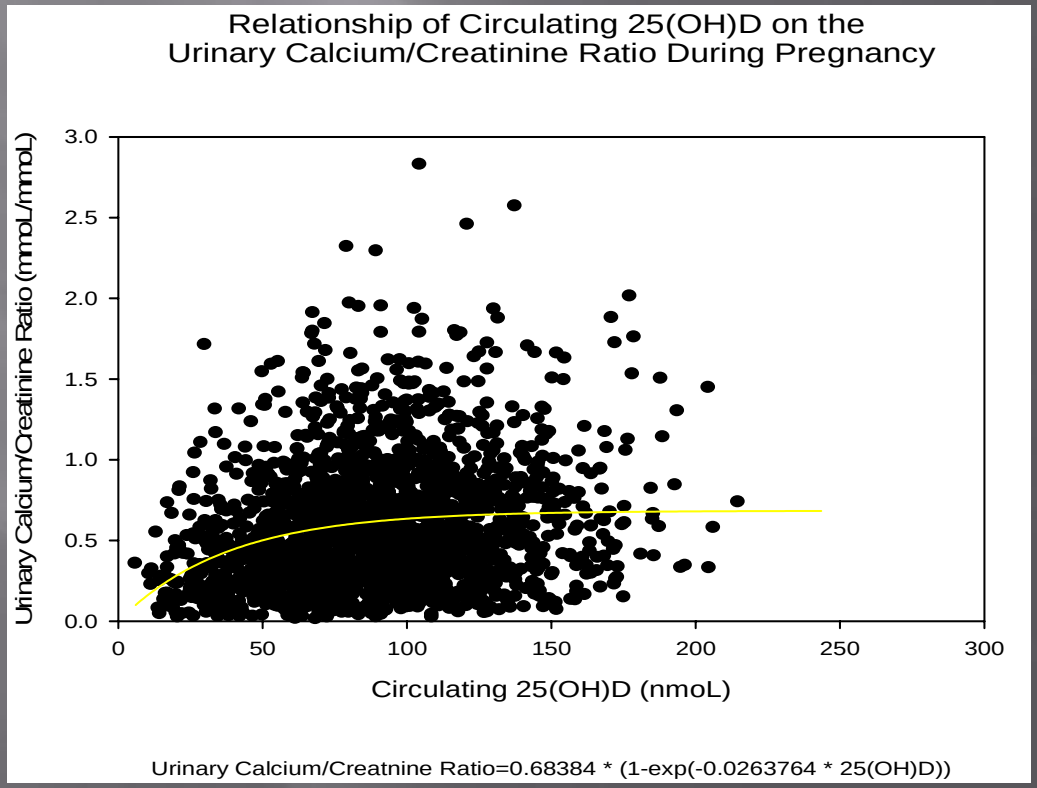
Statistical difference (p-value) in mean circulating $1,25(OH)_2D$ between treatment groups at each week

Treatment Group	12	16	20	24	28	32	36
400 IU vs. 2000 IU	0.3	0.6	0.1	0.06	0.001	0.007	<0.0001
400 IU vs. 4000 IU	0.9	0.1	0.01	0.003	0.001	0.003	<0.0001
2000 IU v 4000 IU	0.4	0.3	0.3	0.3	0.9	0.7	0.8



- 25(OH)D had direct influence on $1,25(OH)_2D$ levels throughout pregnancy ($p<0.0001$)
- This is a saturation curve: inflection point at 40 ng/mL (100 nmol/L) 25(OH)D—the level required to optimize $1,25(OH)_2D$ production.

- Throughout the study, there were no differences between treatment groups or on the basis of circulating 25(OH)D level achieved on any safety measure:
  - Serum Ca, Cr, urinary Ca / Cr ratios (pNS between groups).
- Not a single adverse event was attributed to vitamin D supplementation or total circulating 25(OH)D by the DSMC.



Urine calcium / creatinine ratio vs 25(OH)D

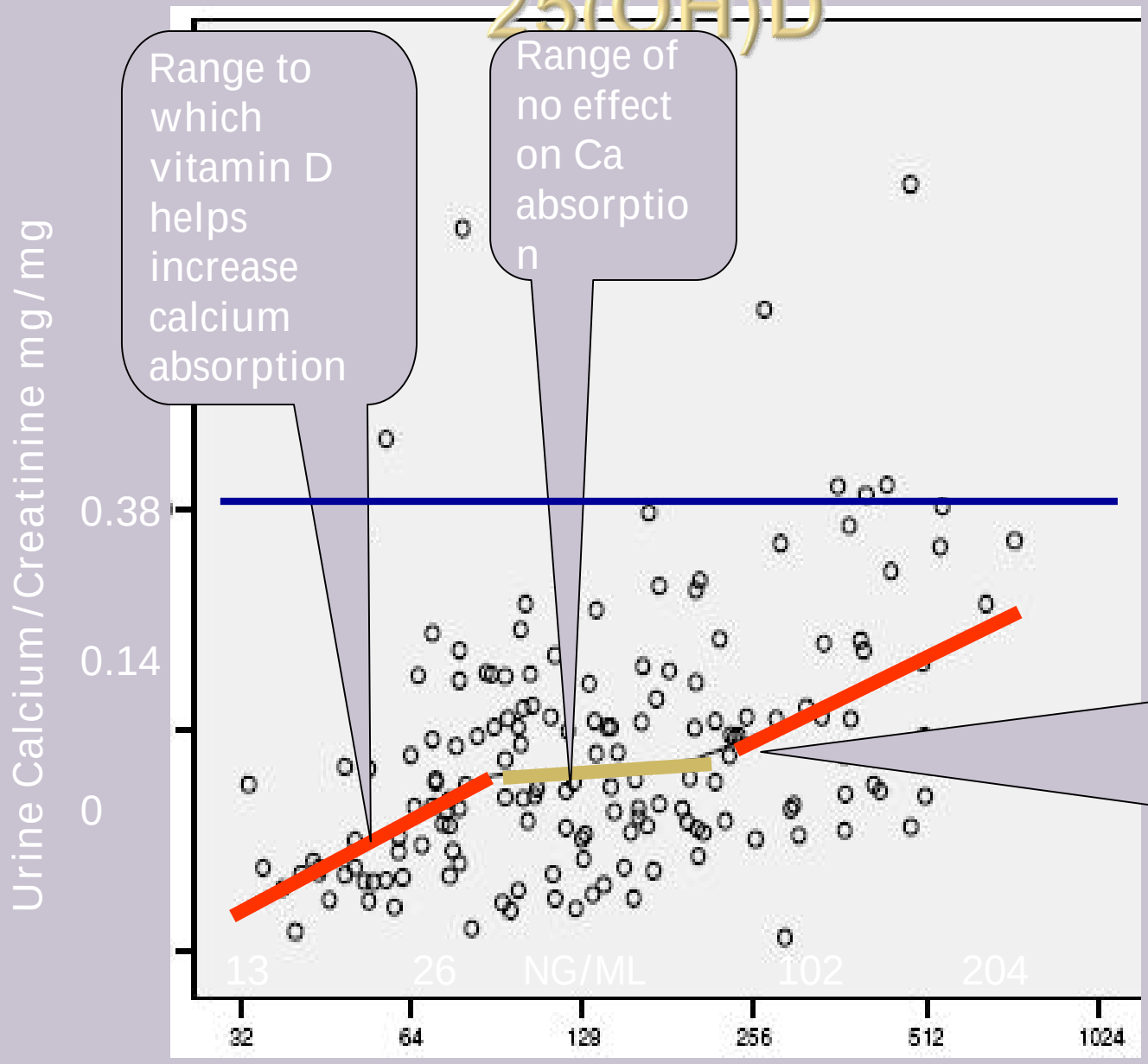
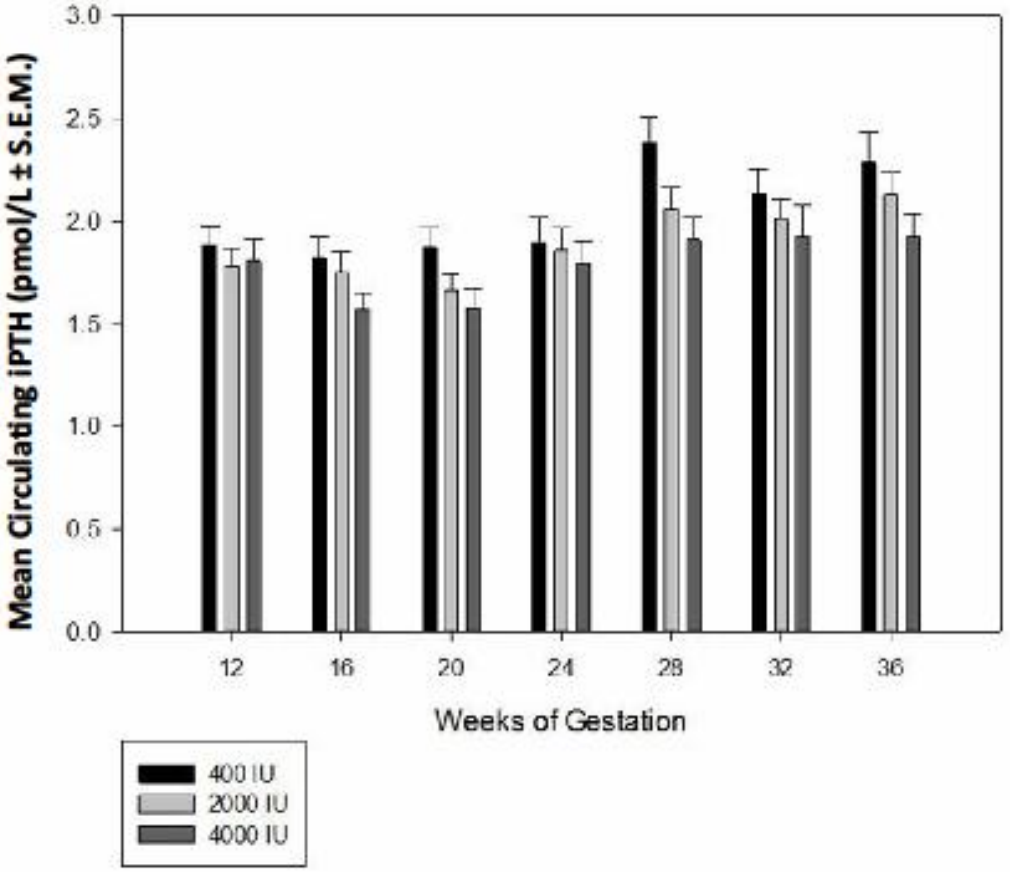


Figure 2d.

Intact Parathyroid Hormone (pmol/L) during Pregnancy by Treatment Group



Statistical difference (p-value) in mean intact PTH between treatment groups at each week

Treatment Group	12	16	20	24	28	32	36
400 IU vs. 2000 IU	0.4	0.6	0.1	0.8	0.049	0.4	0.4
400 IU vs. 4000 IU	0.6	0.06	0.03	0.5	0.006	0.3	0.049
2000 IU v 4000 IU	0.9	0.2	0.5	0.7	0.3	0.6	0.2

Table 2. Pregnancy Characteristics by Vitamin D Supplementation Group

Characteristic	400 IU Group N=111	2000 IU Group N=122	4000 IU Group N=117	p-value	p-value Controlling for Race
Maternal age at delivery (years) (Mean ±SD)	27.4 ±5.7	28.0 ±5.7	27.1 ±5.5	0.49	0.2
Baseline 25(OH)D (nmol/L (Mean ±SD)	61.21± 27.1	57.55 ±22.4	59.82 ±25.4	0.53	0.8
Gestational Age (weeks) at Delivery (Mean ±SD)	38.6 ±2.2	38.8 ±1.8	39.1 ±1.8	0.17	0.1
Birth Weight (grams) at Delivery (Mean ±SD)	3221.8 ±674.9	3360.1 ±585.0	3284.6 ±597.6	0.23	0.2
Mode of Delivery: N (%) Uncomplicated vaginal Assisted vaginal C/S after Labor C/S without Labor Vaginal Primary Cesarean Section	 69 (62.2%) 2 (1.8%) 23 (20.7%) 17 (15.3%) 71 (74.7%) 24 (25.3%)	 81 (66.4%) 4 (3.3%) 19 (15.6%) 18 (14.8%) 85 (79.4%) 22 (20.6%)	 81 (69.8%) 9 (7.8%) 19 (16.4%) 7 (6.0%) 90 (85.7%) 15 (14.3%)	 0.15	 0.046
Previous Preterm Birth N (%)	20 (18.0%)	32 (26.2%)	23 (19.7%)	0.27	0.9
Preterm Birth <37 wks N (%)	9 (8.1%)	5 (4.1%)	7 (6.0%)	0.44	0.5
Preterm Labor<37 wks in this Pregnancy N (%)	16 (14.4%)	22 (18.0%)	14 (12.0%)	0.41	0.4
Preterm Labor/Preterm Birth <37 wks N (%)	23 (20.7%)	24 (19.7%)	20 (17.1%)	0.77	0.4
Gestational Diabetes N (%)	8 (7.2%)	5 (4.1%)	3 (2.6%)	0.25	0.1
Preeclampsia/Eclampsia/Gest Hypertension	9 (8.1%)	6 (4.9%)	3 (2.6%)	0.16	0.05
Infection-Any: N (%) Bacterial Viral Fungal	47 (42.3) 36 (32.4) 8 (7.2) 13 (11.7)	60 (49.2) 44 (36.1) 6 (4.9) 22 (19.0)	44 (37.6) 32 (27.4) 6 (5.1) 13 (11.1)	0.19 0.35 0.71 0.23	0.4 0.3 0.4 0.8
Co-morbidity (PTB) N (%) (infection, PTB, gestational diabetes, preeclampsia/hypertension/help)	63 (56.8)	67 (54.9)	53 (45.3)	0.17	0.06
Co-morbidity (PTL/PTB) N (%) (infection, PTL/PTB <37 weeks, gestational diabetes, preeclampsia/hypertension/HELLP)	70 (63.1)	72 (59.0)	59 (50.4)	0.14	0.03
Pill Count--Pills taken:pills issued (median)	0.47	0.49	0.50	0.70	0.9

Table 3. Number and Percentage of Small-for-Gestational-Age (SGA) Infants by Vitamin D Treatment Group[a]

Racial group	Number of subjects	400 IU/d	2,000 IU/d	4,000 IU/d
Caucasian	111	1(0.9%)	0	0
African American	97	6 (6.2%)	4 (4.1%)	5 (5.2%)
Hispanic	137	6 (4.4%)	1 (0.7%)	4(2.9%)

[a] Data derived from Hollis et al [1].

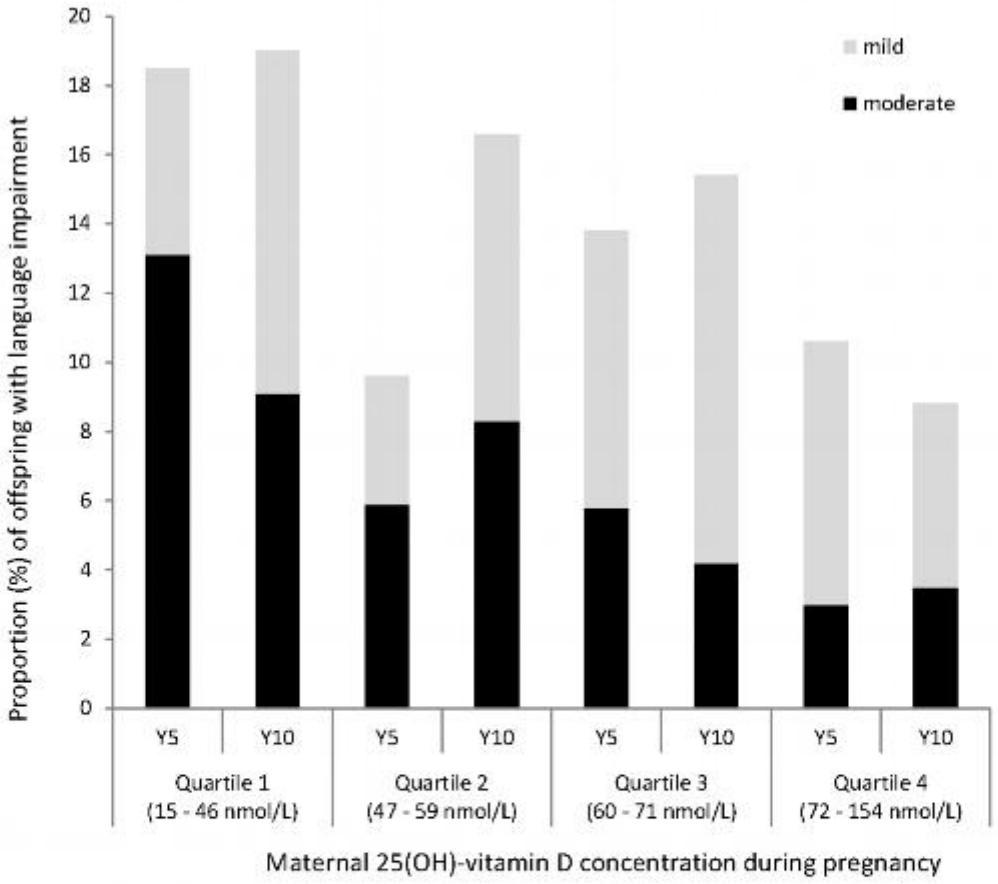


Figure 1. The proportion of offspring with mild or moderate-severe language impairment at 5- (Y5)* and 10-years (Y10)† of age according to maternal serum 25(OH)-vitamin D levels at 18 weeks pregnancy.

*N: Quartile 1 = 130, Quartile 2 = 136, Quartile 3 = 136, Quartile 4 = 132;

†N: Quartile 1 = 121, Quartile 2 = 121, Quartile 3 = 119, Quartile 4 = 113;

Hypotheses—were they correct?

- H_1: The prenatal maternal nutritional requirement for vitamin D, that is, the amount required to elevate circulating 25(OH)D, will be substantially greater in darkly pigmented pregnant women due to limited cutaneous synthesis of vitamin D_3.
  - YES!!!
- H_2: High-dose (2,000 or 4,000 IU / day) vitamin D supplementation of pregnant mothers will provide sufficient antirachitic activity to prevent hypovitaminosis D in the pregnant mother and her fetus, regardless of ethnicity and sunlight exposure of the subject. Further, this supplementation level will be safe and efficacious without any adverse side effects or health consequences in the mother or fetus.
  - YES!!!

Thrasher Research Fund Community- Based Study in Columbia , SC

- Diverse group of women randomized to 2000 or 4000 IU vitamin D_3 / day irrespective of baseline 25(OH)D at <16 weeks' gestation:
- 257 women were enrolled; 157 women completed the study;
- Confirms NIH / NICHD study findings
- No adverse events associated with vitamin D supplementation

Secondary Endpoints

- Lower rate of infection (OR 0.73 per 10 ng / mL 25(OH)D increase, CI 0.604-0.893, p=0.002) among those with higher 25(OH)D levels; this association persisted after controlling for race.
- Analysis of complications during pregnancy as a function of change in 25(OH)D from baseline and the level one month prior to delivery
  - preterm labor / preterm birth
  - rates of infection were inversely related to measures of vitamin D status
    - effect persisted even after controlling for race.
  - No adverse events were associated with vitamin D supplementation or total circulating 25(OH)D.

Figure 1

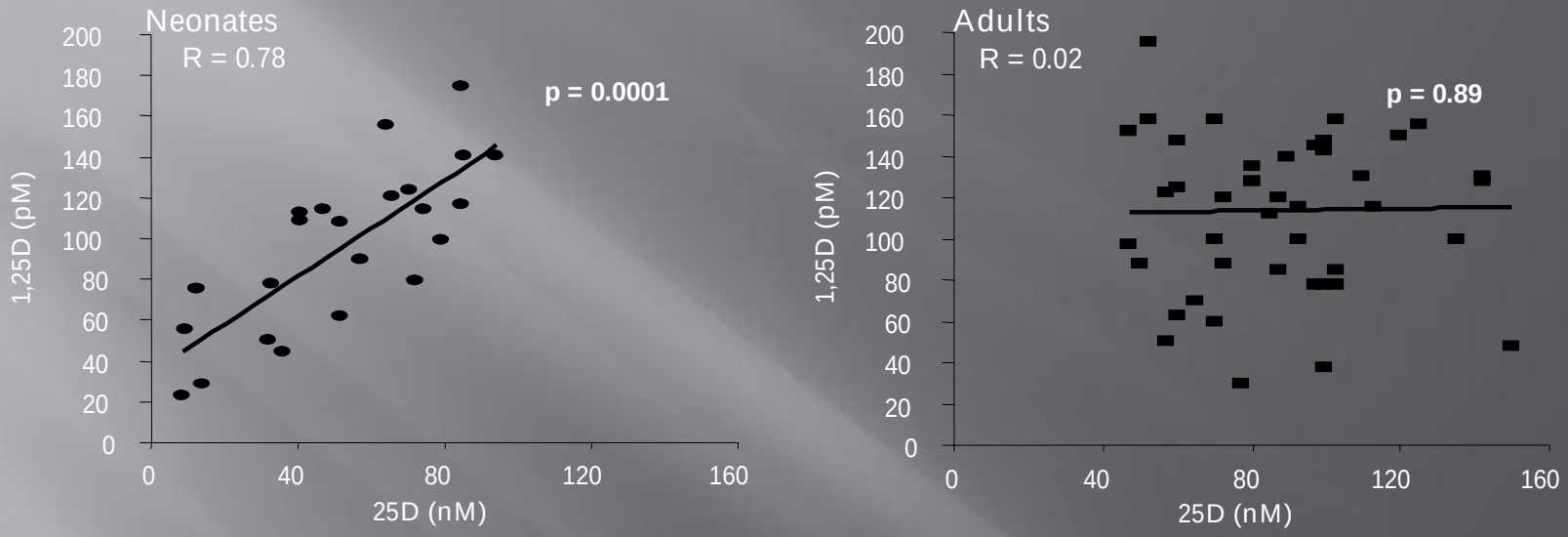
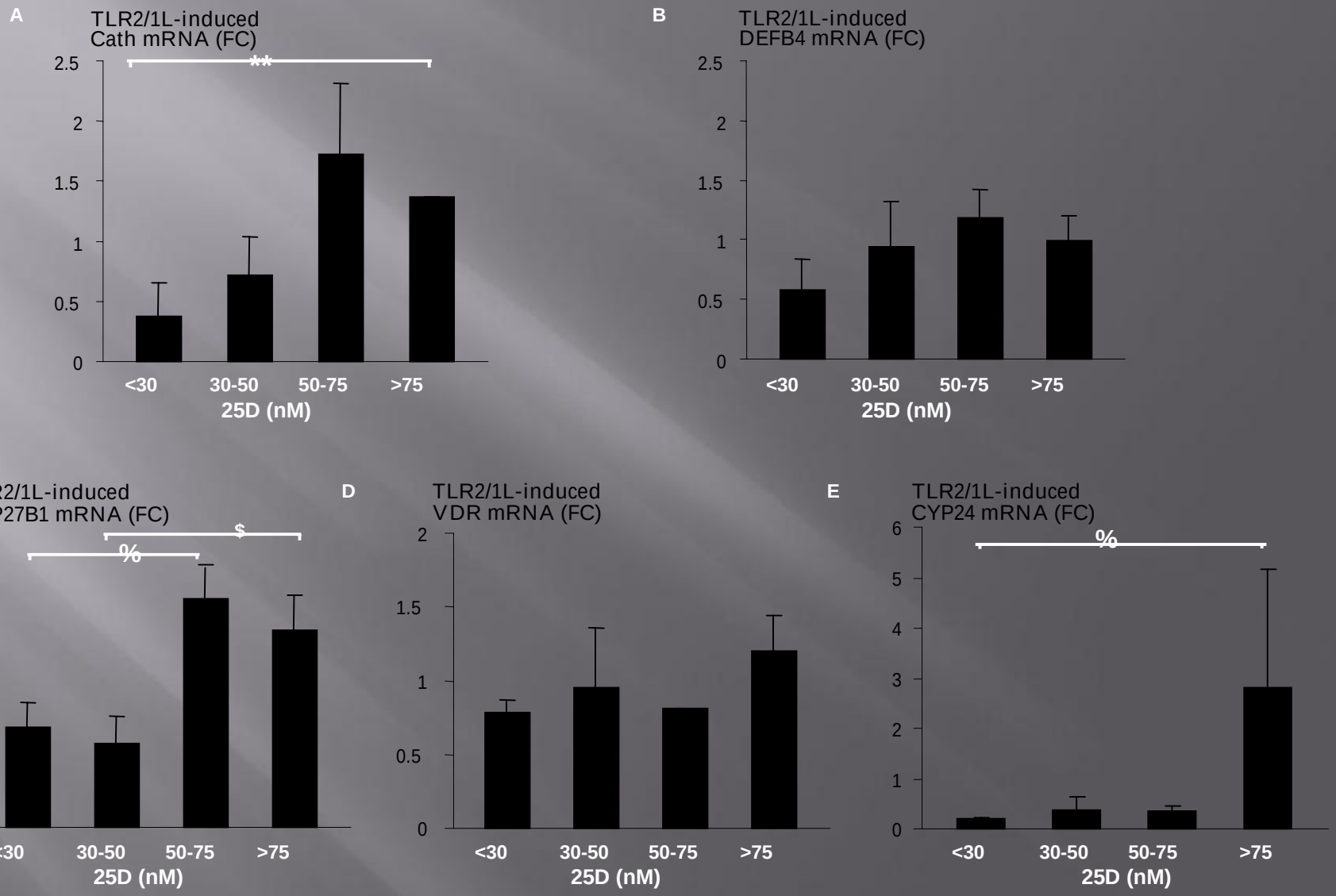

Neonates
R = 0.78
p = 0.0001
1,25D (pM)
25D (nM)
0
20
40
60
80
100
120
140
160
180
200
0
40
80
120
160
Adults
R = 0.02
p = 0.89
1,25D (pM)
25D (nM)

Figure 3



**p < 0.01

$p = 0.04

%p = 0.02

Conclusions Regarding Safety from both NIH and Thrasher Pregnancy Studies

- Vitamin D supplementation with 4,000 IU vitamin D / day for pregnant women was safe and effective in achieving vitamin D sufficiency in a racially diverse group.
- To normalize vitamin D metabolism in the pregnant woman, a circulating 25(OH)D level of at least 40 ng / mL (100 nmol / L) is required.
- In both studies, higher maternal circulating 25(OH)D was associated with lower risk of co-morbidities of pregnancy.
- Therefore, we recommend for all pregnant women:
  - Checking 25(OH)D levels at the start of pregnancy
  - Achieve a 25(OH)D level of at least 40 ng / mL (100 nmol / L) for optimal conversion of to $1.25(OH)_2D$
    - This can be achieved through vitamin D supplementation of 4000 IU / day starting at 12 weeks' gestation

Lactation

- 5 year NIH-sponsored RCT
- Mothers receive 400, 2400, or 6400 IU / day vitamin D3
- Infants receive 400 IU / day or placebo
- Treatment had to sustain a circulating level of 25(OH)D >20 ng / ml in the nursing infant

Maternal Supplementation with 400 IU vitamin D_3/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 D_3/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

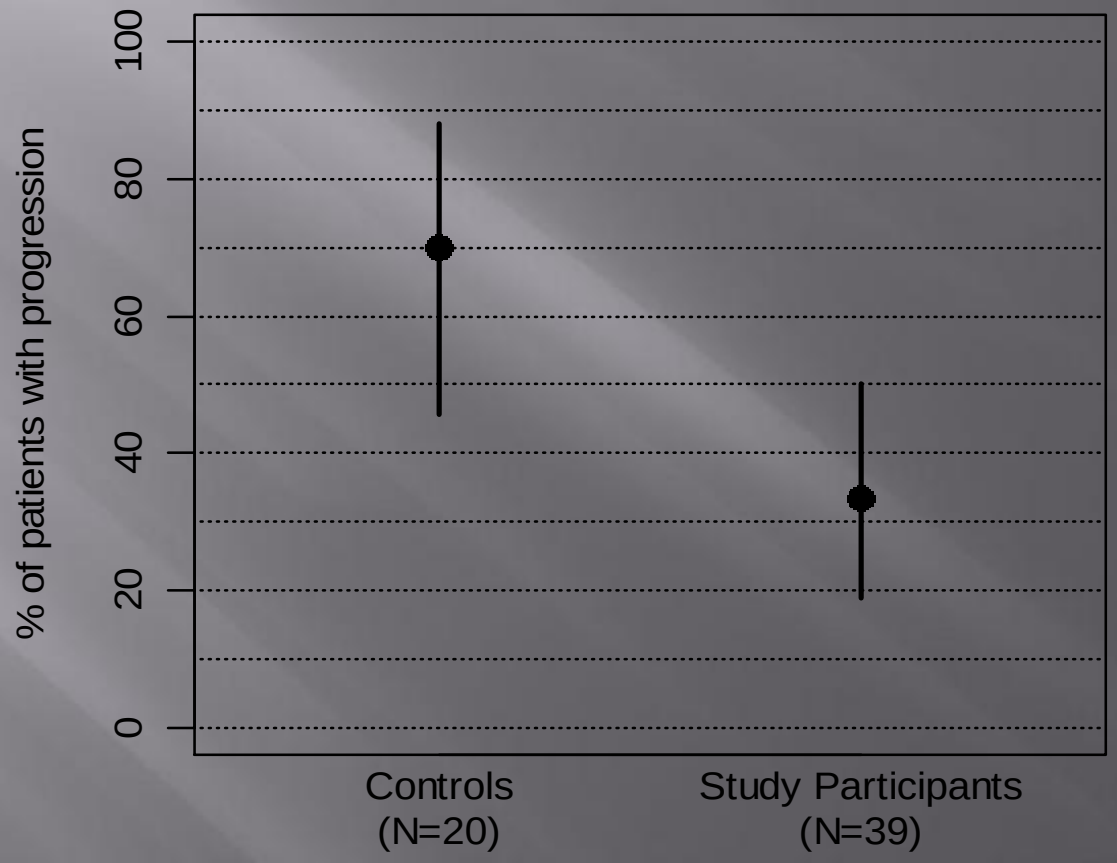
Lactation Trial is ongoing

- However, 2 years into the study the 2400 IU / day arm was terminated, as directed by the DSMB, because it failed to maintain a minimal circulating 25(OH)D level in the nursing infant.

MUSC Prostate Cancer Intervention Trial

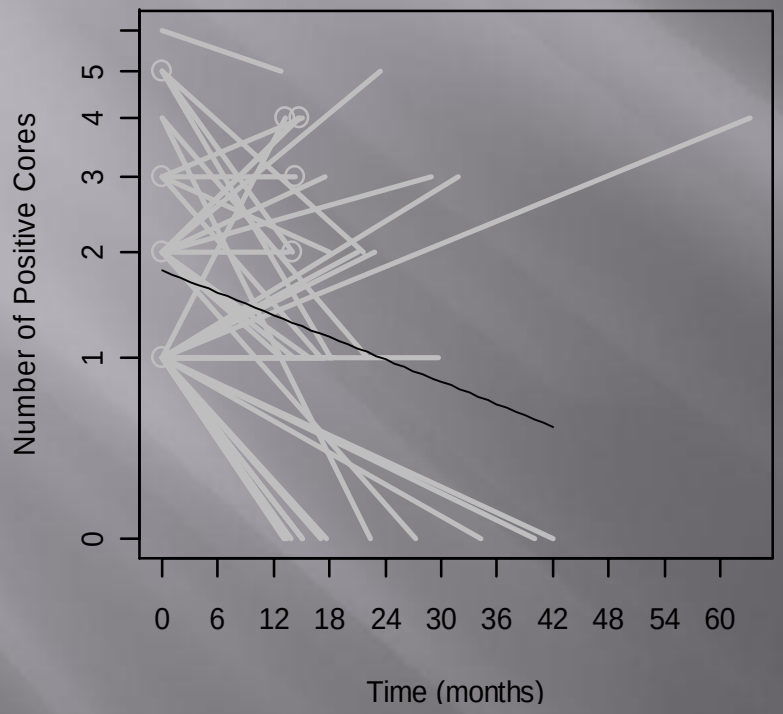
- Stage 1 Prostate Cancer Patients, PSA < 10 and Gleason Score <6.
- These Guys are Watch and Wait.
- One Year Trial Getting 4,000 IU/d Vitamin D_3.
- PSA was Measured.
- Prostate Biopsy at Diagnosis and Upon Exiting the Study.

Percent of Prostate Cancer Subjects with Increase in Positive Cores at Repeat Biopsy

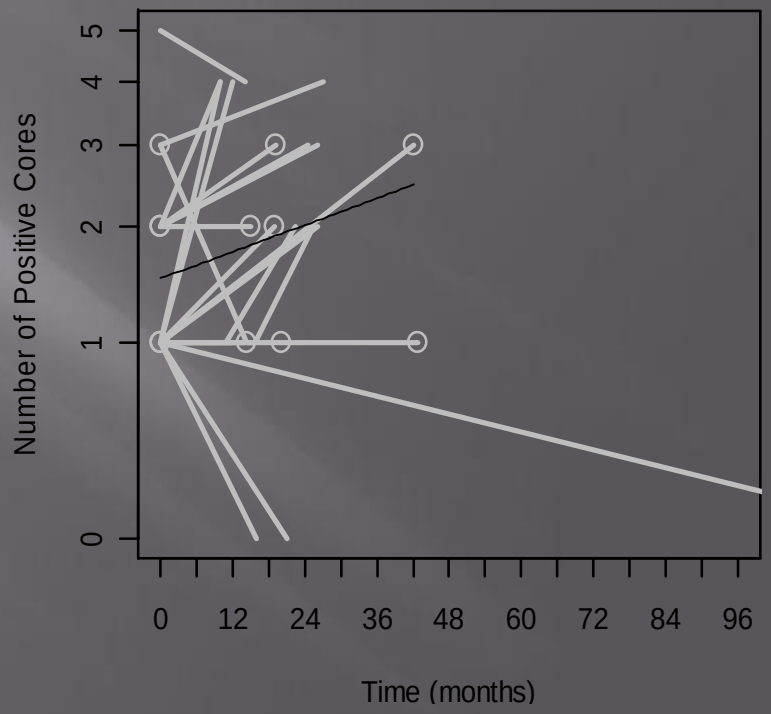


Positive cores at repeat biopsy compared to baseline in supplementation and control groups

4000 IU / day Group



Control Group



Effect of Vitamin D Supplementation on Men with Early Prostate Cancer: One Year Follow-Up

Characteristic	No Change n=1 (3.0%)	Progression n=12 (36%)	Improvement N=20 (61%)
Baseline 25(OH)D (ng/mL)	18.2	31.9 ± 17.3	30.4 ± 12.7
Exit 25(OH)D (ng/mL)	49.2	69.8 ± 14.7	65.3 ± 15.1
Baseline PSA (ng/mL)	2.44	4.63 ± 1.70	4.55 ± 1.94
Exit PSA (ng/mL)	2.80	5.57 ± 2.37	4.81 ± 2.96
Baseline Positive Cores	1.0	1.91 ± 1.16	1.95 ± 1.50
Exit Positive Cores	1.0	3.33 ± 0.98	0.65 ± 1.22

Patient Category	Baseline Positive Cores	Exit Positive Cores	Disease Change from Baseline
Open-Label (n=32)	1.94 ± 1.37	1.66 ± 1.73	No change: 3% Progression: 36% Improvement: 61%
Open-Label Matched Controls (n=11)	1.18 ± 1.9	1.91 ± 1.0	No change: 10% Progression: 80% Improvement: 10%
Unmatched on Active Surveillance (n=19)	0.26 ± 0.93	2.42 ± 2.19	No change: 0% Progression: 100% Improvement: 0%

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Thank You