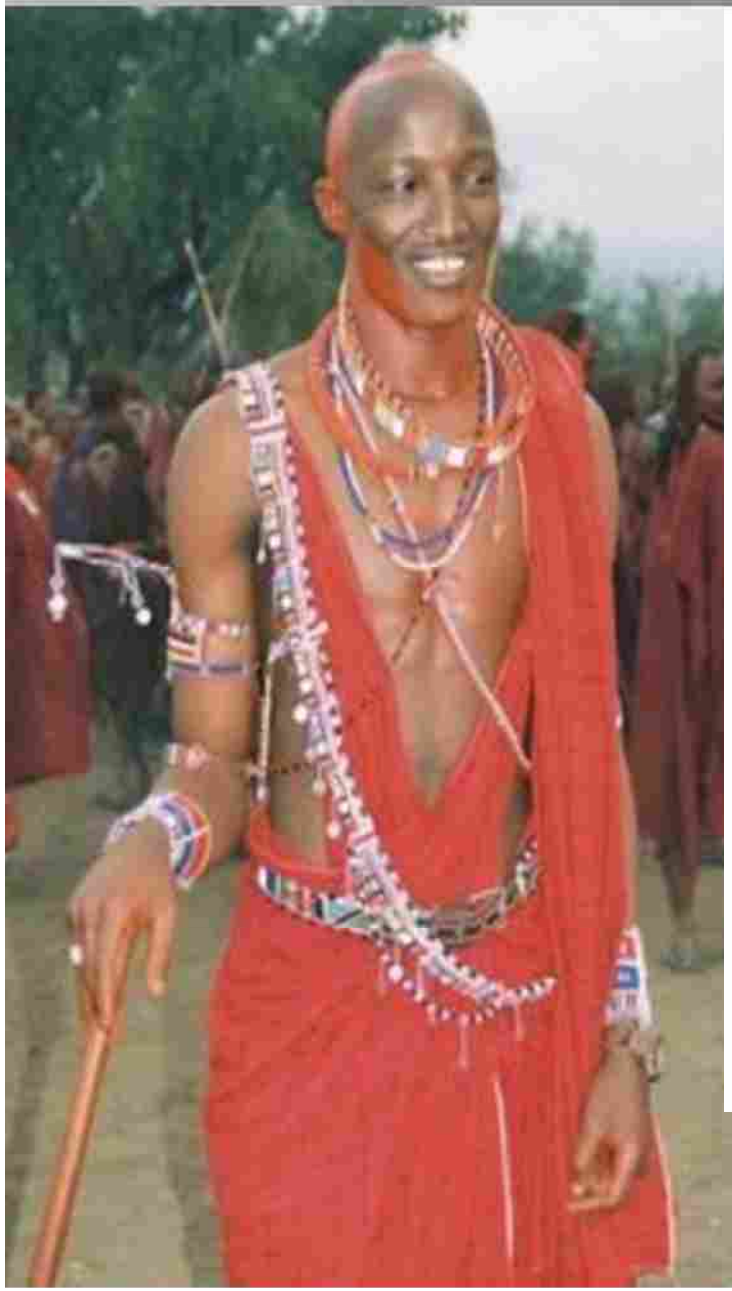
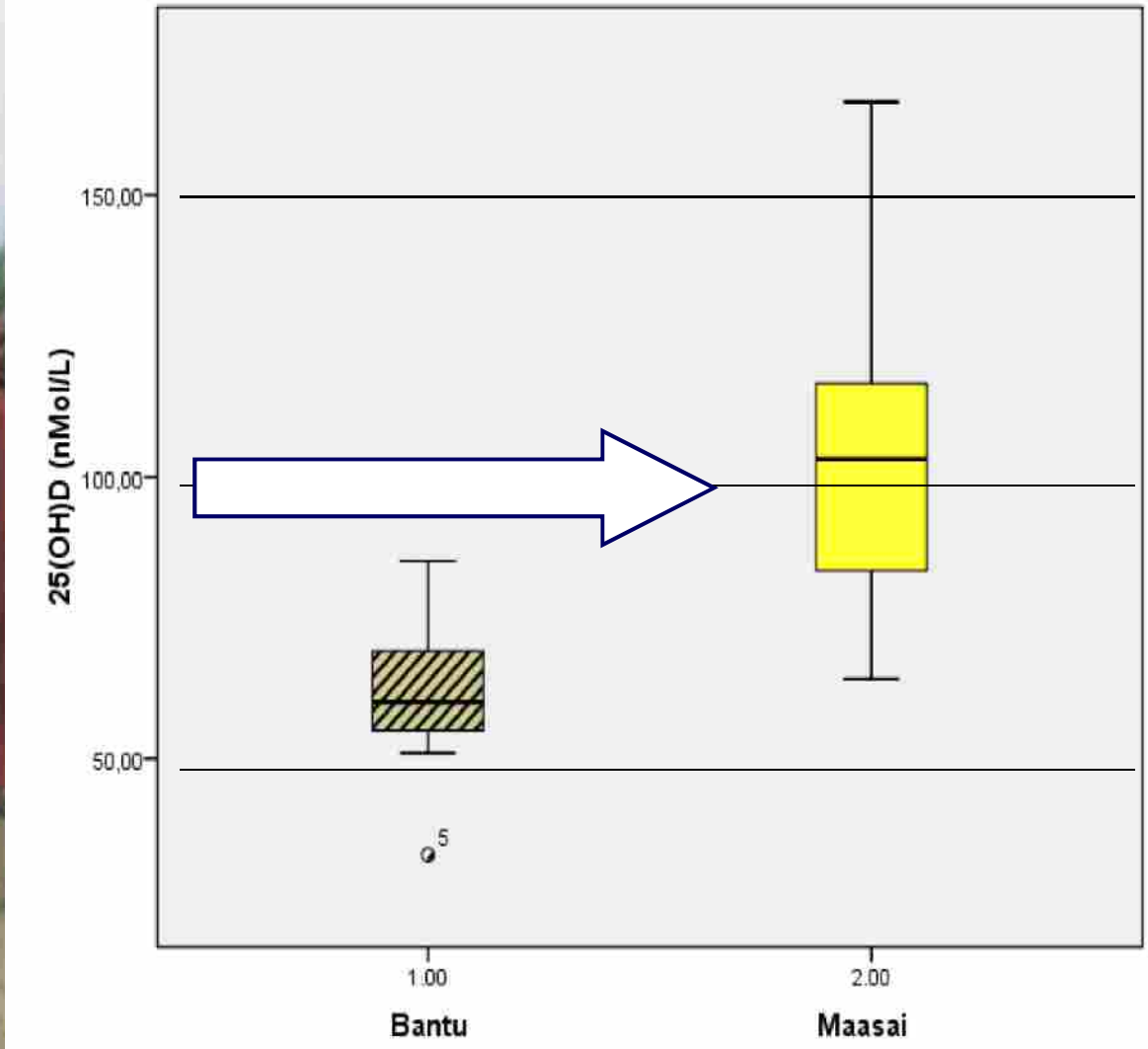
THE VITAMIN D REQUIREMENT DURING PREGNANCY AND LACTATION

Bruce W. Hollis, Ph.D. and Carol L. Wagner, M.D.

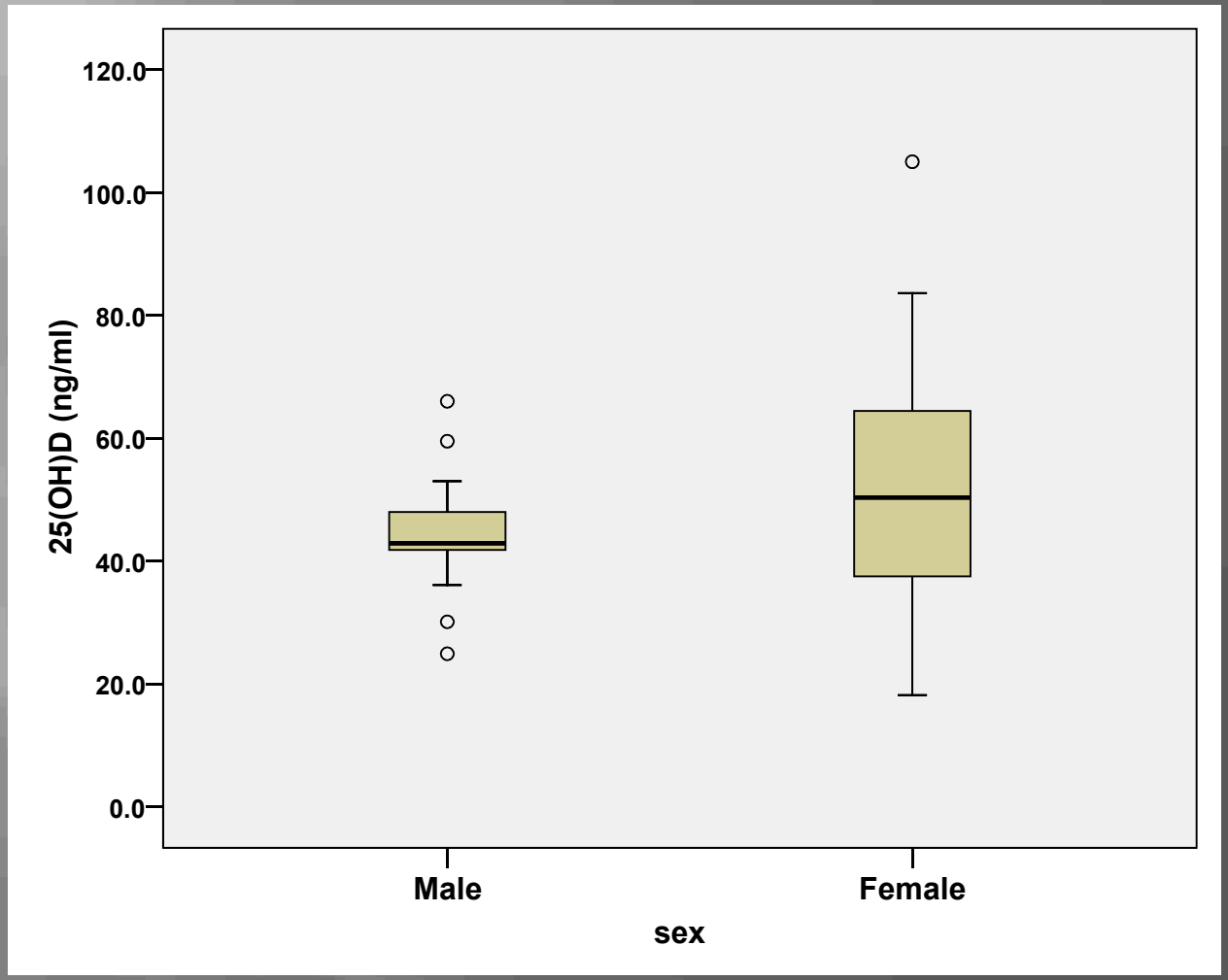
MAASAI MEDIAN 25(OH)D = 104 NMOL/L





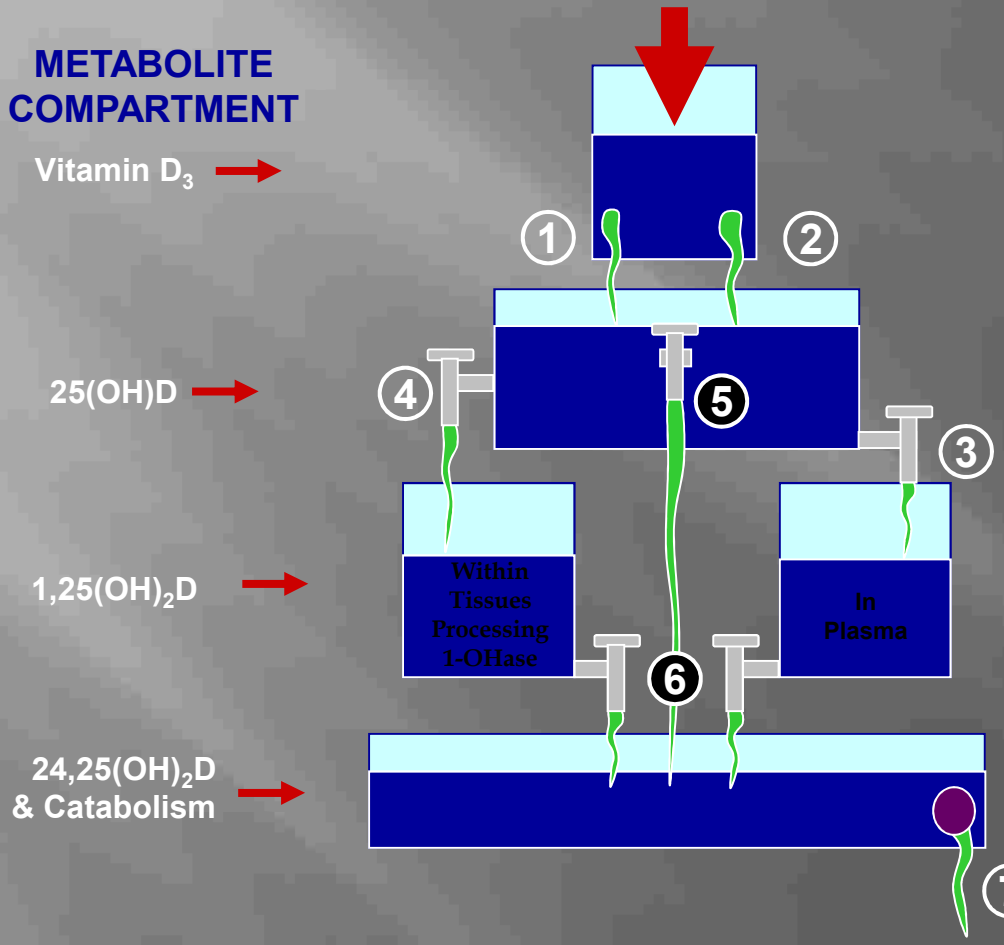
Luxwolda and Muskiet ,
submitted manuscript

Laramie, WY Athletes: No Supplementation



Metabolism of Vitamin D Under Conditions of Adequate Vitamin D Supply

High/Normal Input of Cholecalciferol from diet or UVB



LEGEND

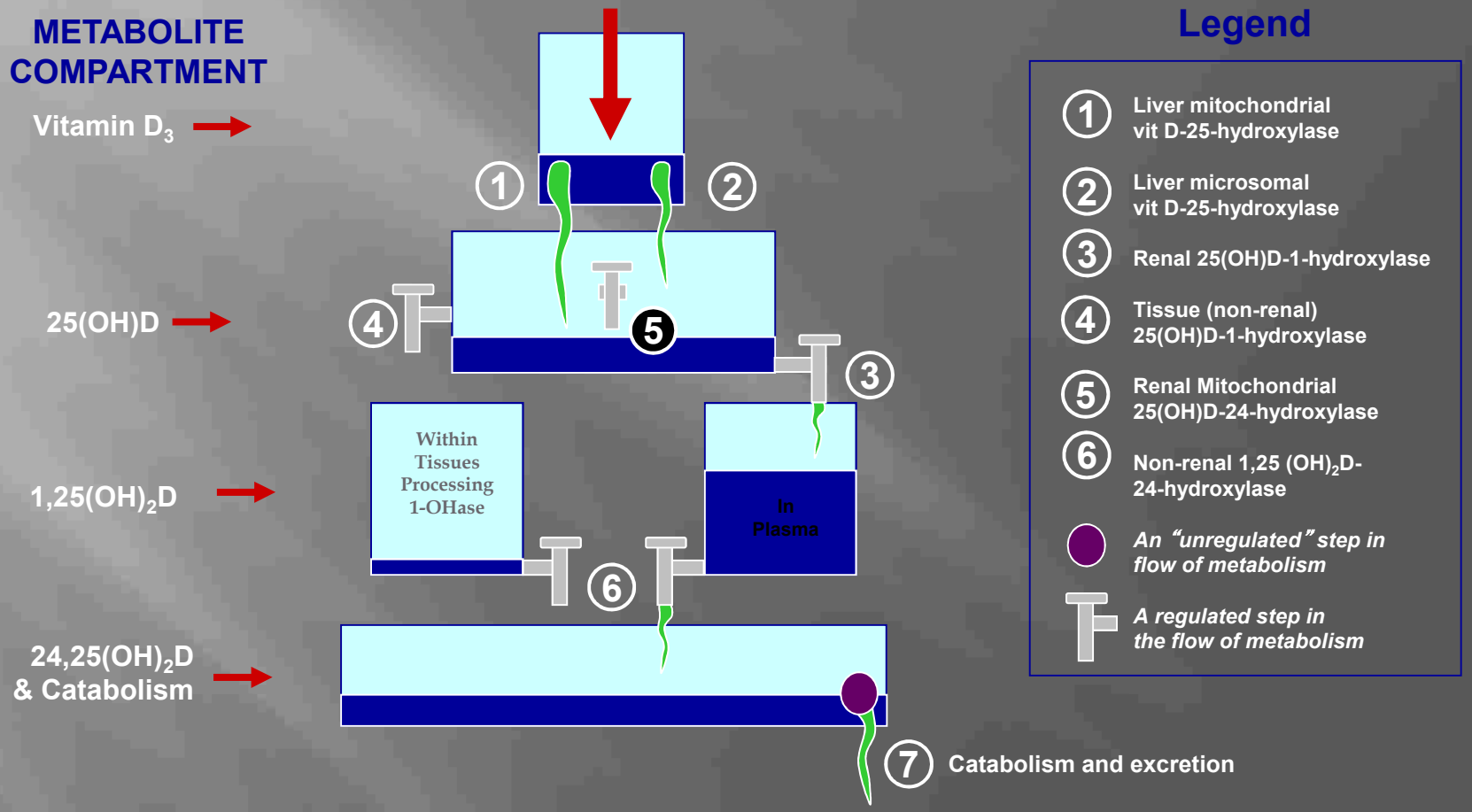
1. Liver mitochondrial vit D-25-hydroxylase
2. Liver microsomal vit D-25-hydroxylase
3. Renal 25(OH)D-1-hydroxylase
4. Tissue (non-renal) 25(OH)D-1-hydroxylase
5. Renal Mitochondrial 25(OH)D-24-hydroxylase
6. Non-renal $1,25(OH)_2D$-24-hydroxylase

An "unregulated" step in flow of metabolism

A regulated step in the flow of metabolism

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.

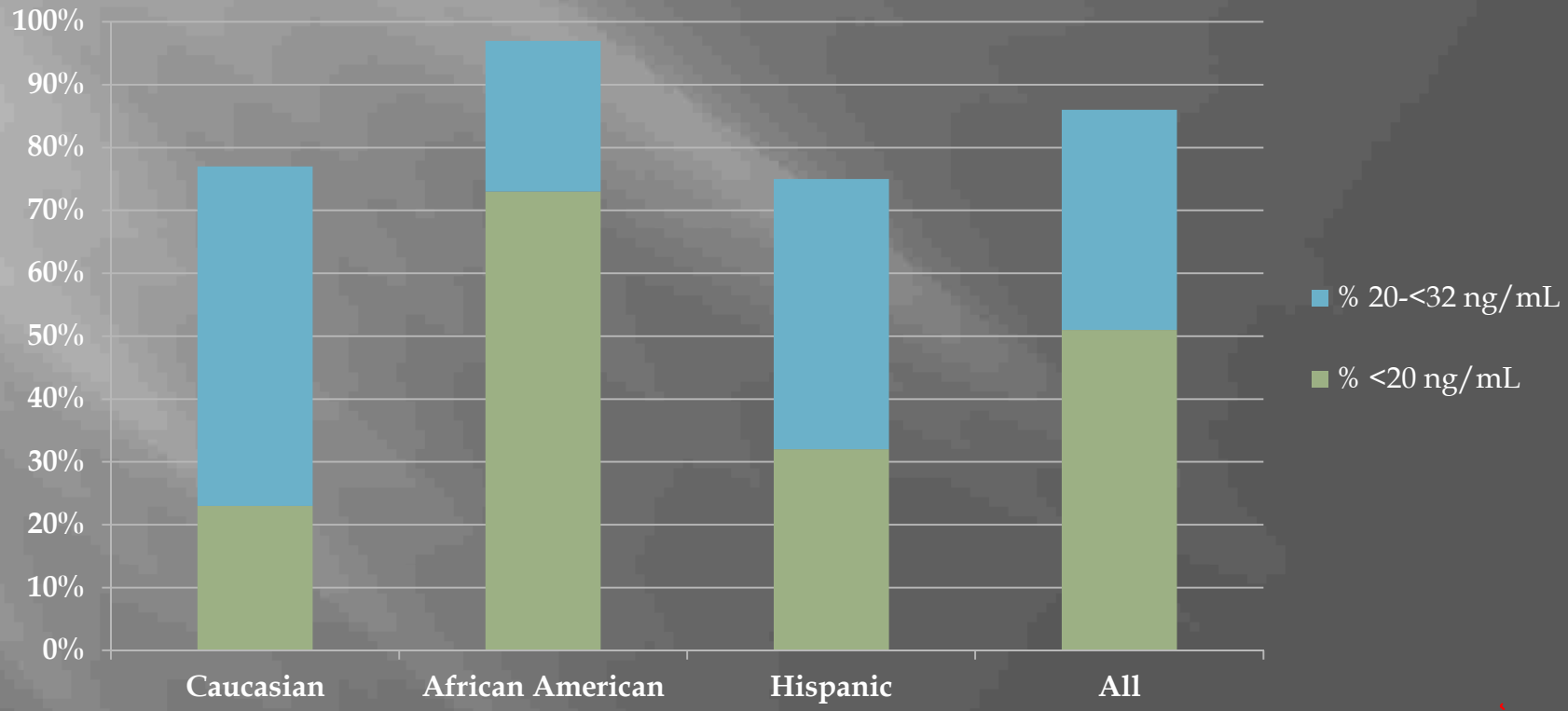
Metabolism of Vitamin D Under Conditions of Low Vitamin D Supply



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

Baseline Circulating 25(OH)D Levels



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?

Objective of 2 Vitamin D RCT Trials Conducted in S.C.

- Evaluate the safety and effectiveness of high dose vitamin D supplementation in achieving vitamin D sufficiency.

NIH 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.

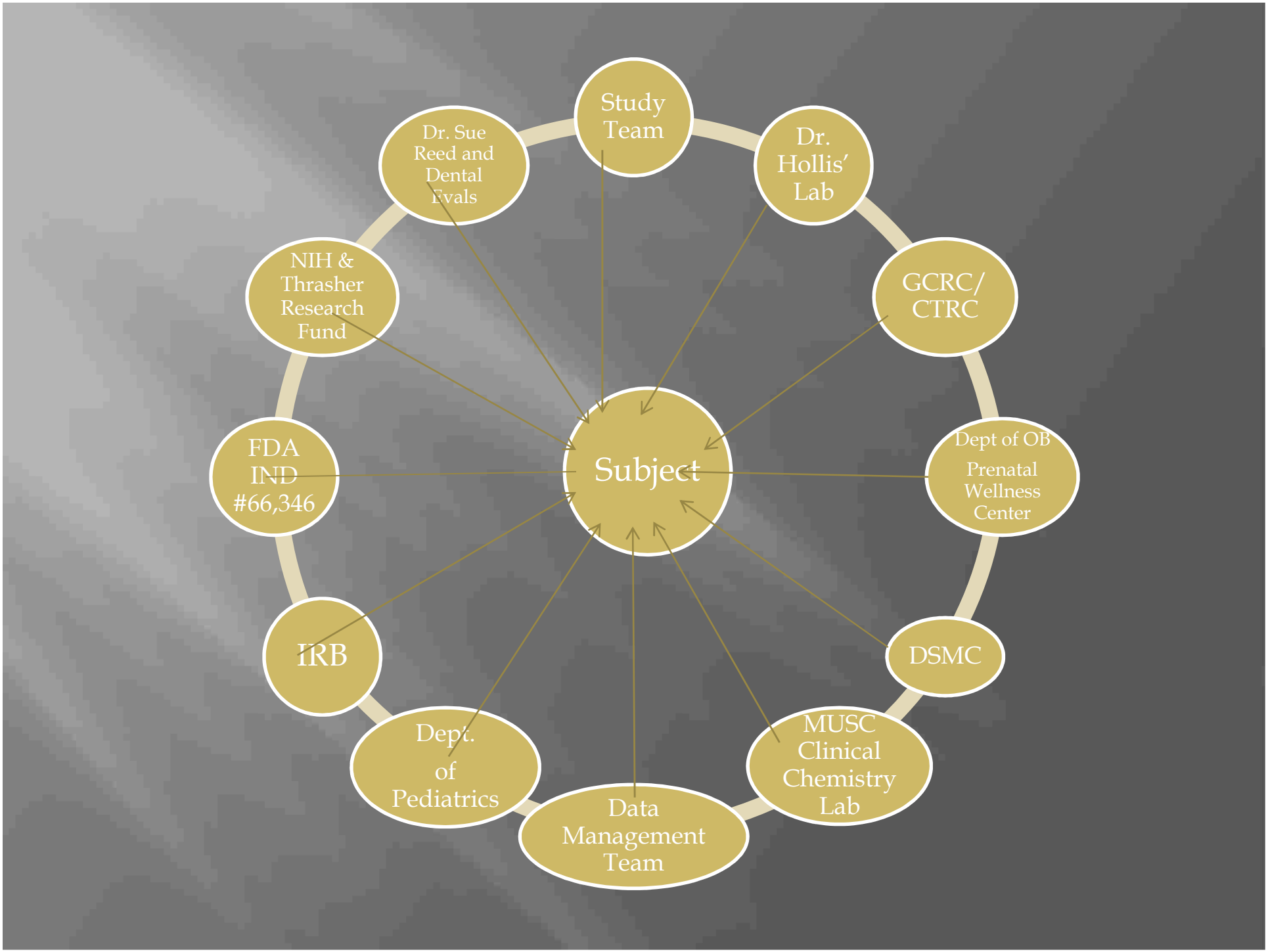
Hypotheses

- **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.

- **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.

Methods and Study Design

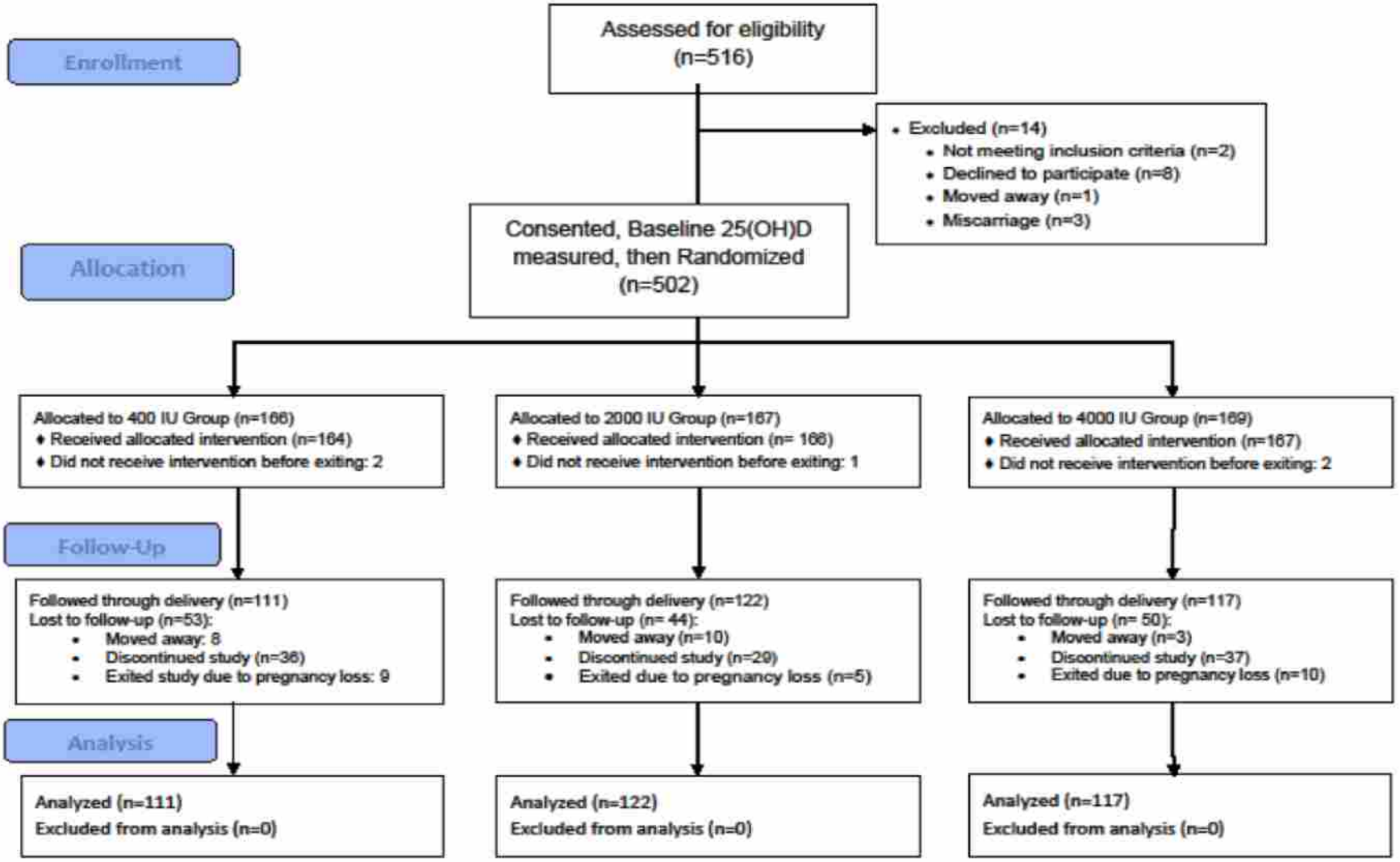
- Study Design: randomized control, double-blind placebo study of vitamin D supplementation.
- Women <16 weeks' singleton gestation eligible for participation in the study; those with any underlying diabetes or hypertension not eligible to participate
- Baseline 25(OH)D levels measured
  - Women with 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$(OH)_2$D; 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

Figure 1. Flow Diagram of Pregnancy Study



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

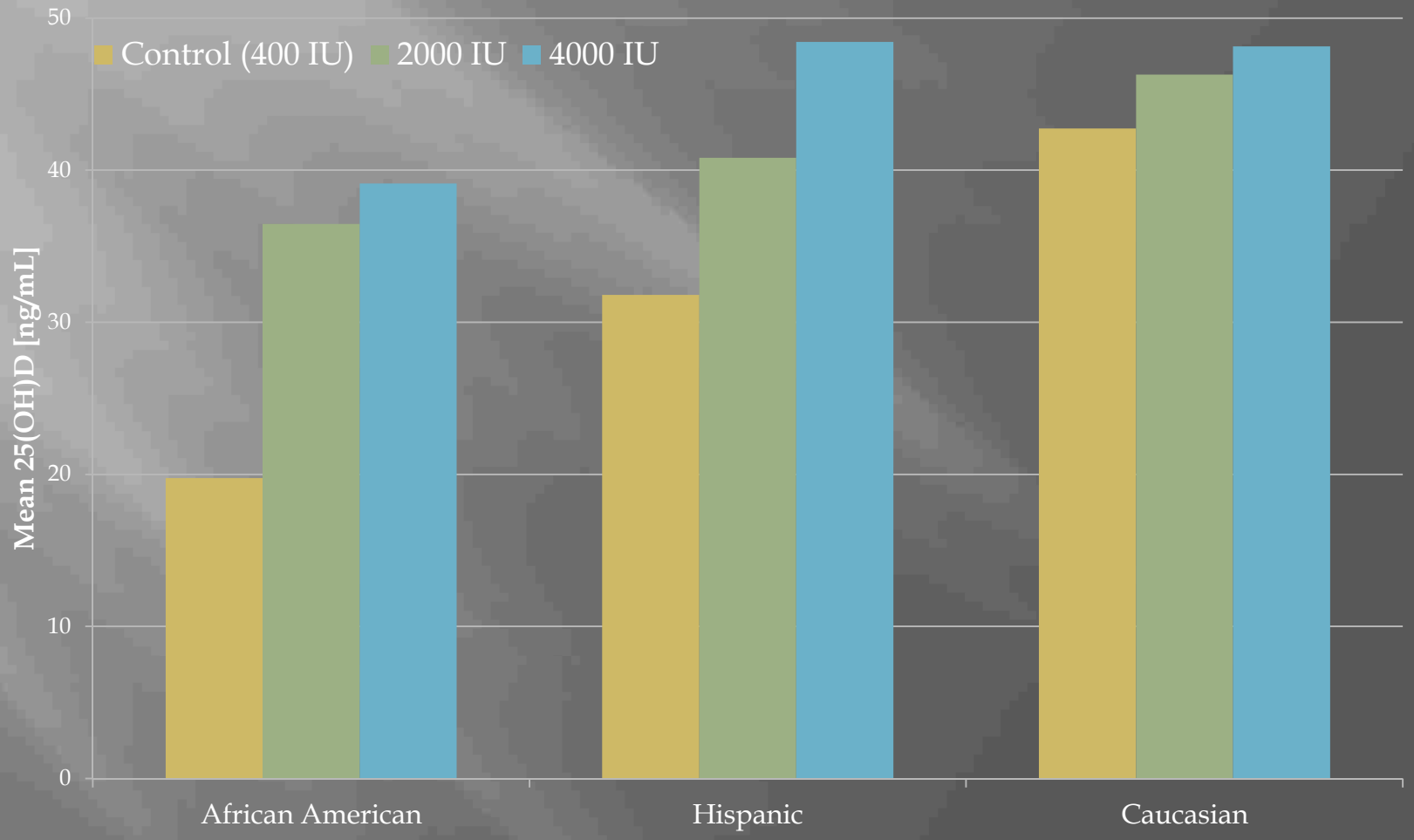
Subjective Health Rating Scale from 1 (poor) -10 (excellent) (Median) (Range)	**9 5-10**	**10 5-10**	**10 1-10**	**0.4**
Planned Pregnancy, N (%)	59 (54.6)	61 (50.4)	59 (50.4)	0.8
BMI: N (%) ≤30 > 30	78 (70.3) 33 (29.7)	87 (71.3) 35 (28.7)	89 (76.1) 28 (23.9)	0.6
Season at study entry: N (%) April - 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

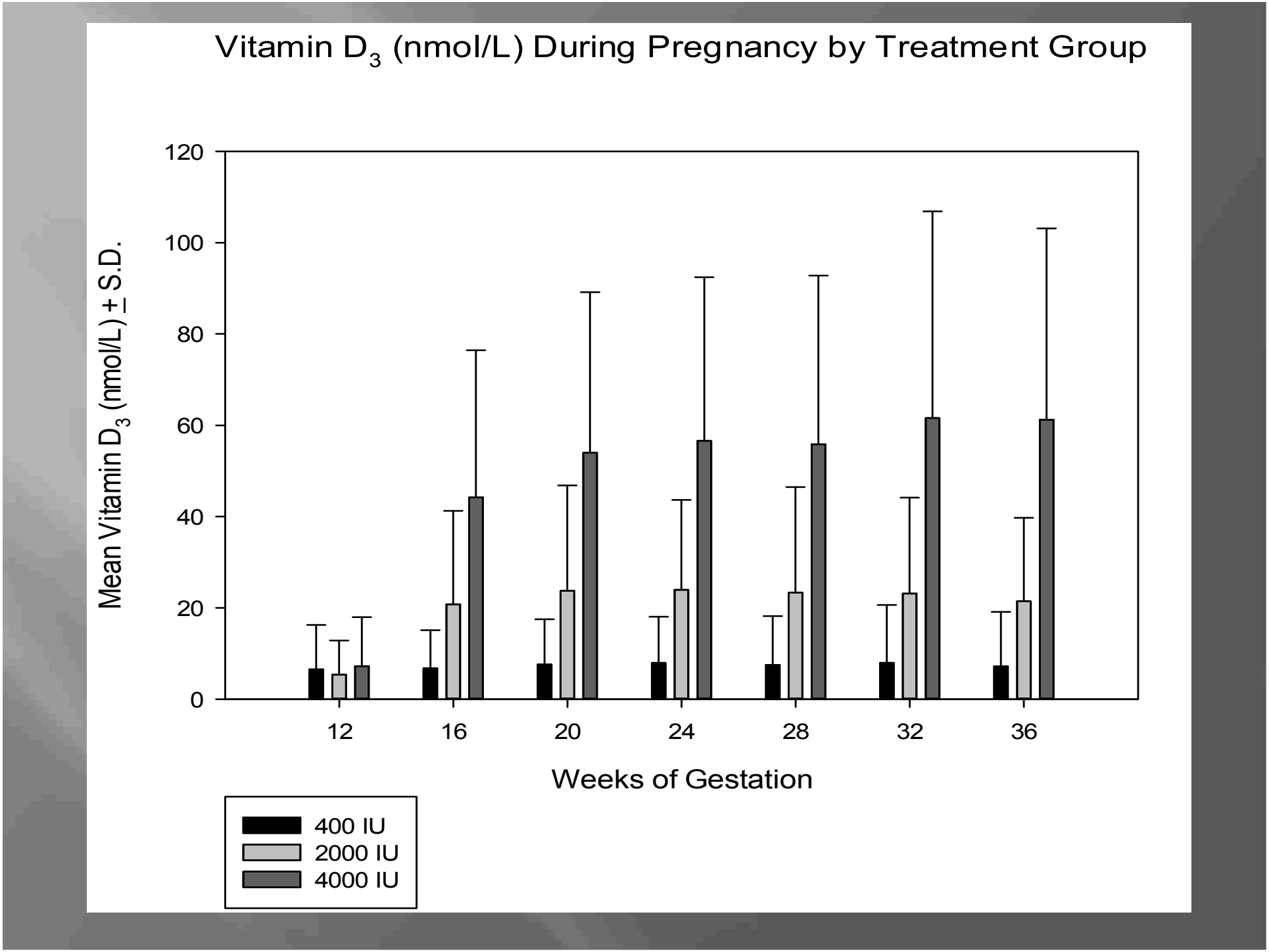
Mean 25(OH)D

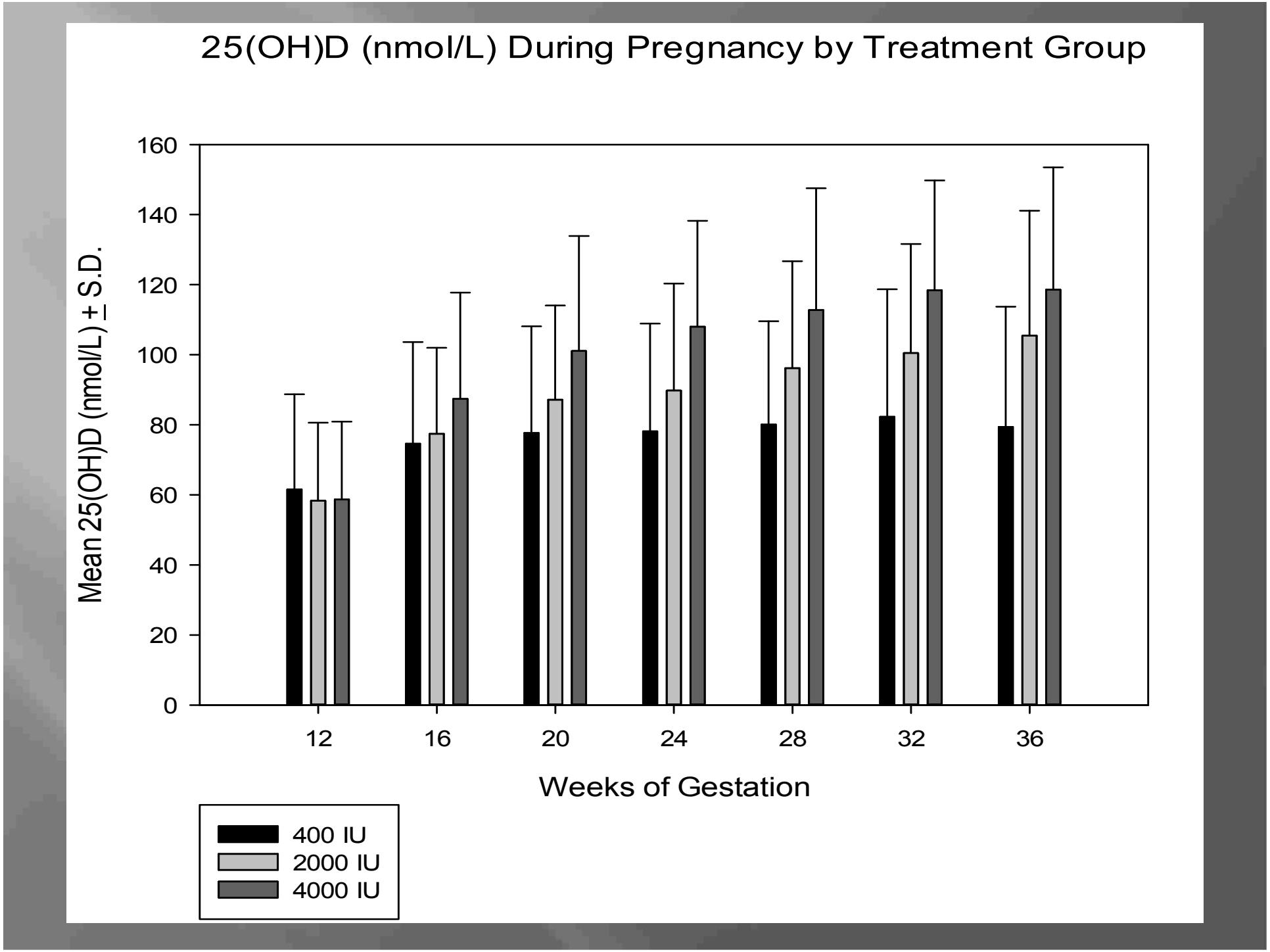
- Intent-to-Treat Analyses:
  - Varied by treatment group at delivery
  - Varied as chronic status measured by area under the curve
  - 1-month before delivery
    - significantly different between:
      - Control *vs.* 2000 IU group ($p<0.0001$)
      - Control vs. 4000 IU group ($p<0.0001$)
     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

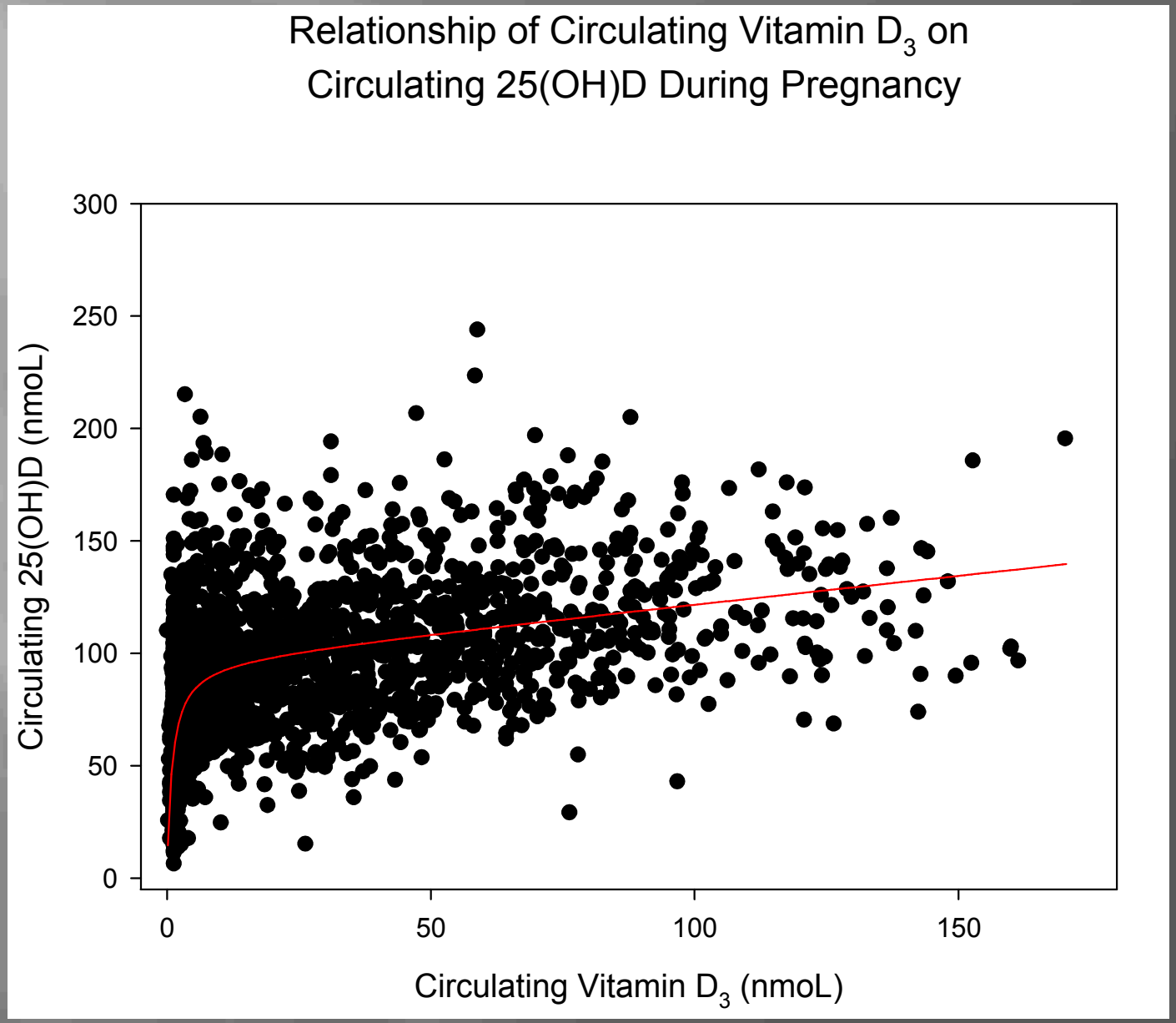
Characteristic	400 IU Group N=111	2000 IU Group N=122	4000 IU Group N=117	p-value
Maternal age at delivery (years), Mean ± SD	27.4 ±5.7	28.0 ±5.7	27.1 ±5.5	0.49
Baseline 25(OH)D (nmoL), Mean ± SD	61.21± 27.1	57.55 ±22.4	59.82 ±25.4	0.53
Mode of Delivery: N (%) Uncomplicated vaginal Assisted vaginal C/S after Labor C/S without Labor Vaginal, Any type 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
Pill Count Pills taken: pills issued, Median	0.74	0.74	0.75	0.9
Gestational Age (weeks) at Delivery Mean ± SD	38.6 ±2.2	38.8 ±1.8	39.1 ±1.8	0.17
Birth Weight (grams) at Delivery, Mean ± SD	3221.8 ±674.9	3360.1 ± 585.0	3284.6 ± 597.6	0.23
Neonatal Total Circulating 25(OH)D, nmol/L Mean ± SD	45.5 ± 10.1	57.0 ± 9.8	66.3 ± 10.3	<0.0001

Mean Circulating 25(OH)D at 1 Month Prior to Delivery by Race/Ethnicity

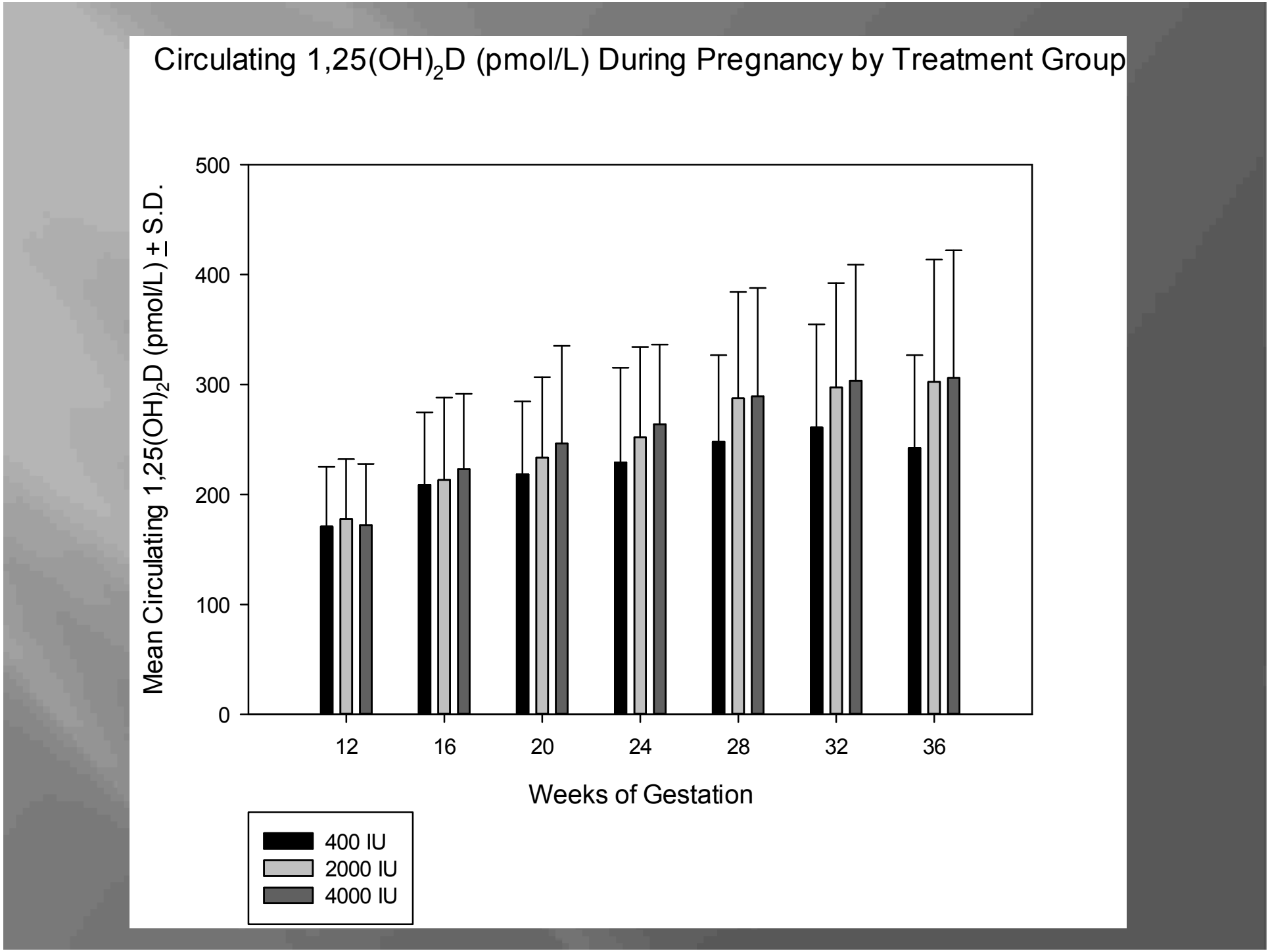


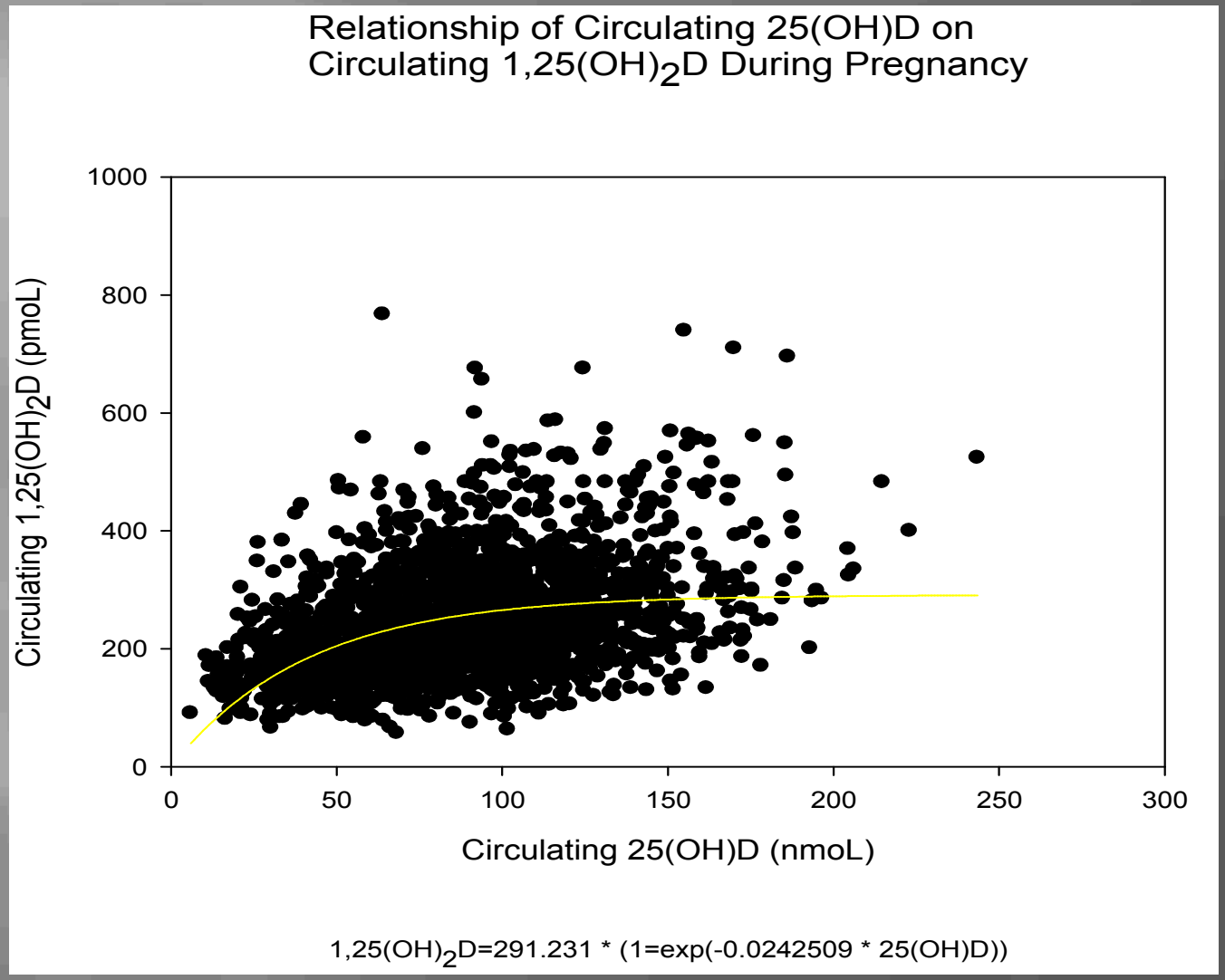

Vitamin D_3 (nmol/L) During Pregnancy by Treatment Group
Mean Vitamin D_3 (nmol/L) ± S.D.
120
100
80
60
40
20
0
12
16
20
24
28
32
36
Weeks of Gestation
400 IU
2000 IU
4000 IU


25(OH)D (nmol/L) During Pregnancy by Treatment Group
Mean 25(OH)D (nmol/L) ± S.D.
160
140
120
100
80
60
40
20
0
12
16
20
24
28
32
36
Weeks of Gestation
400 IU
2000 IU
4000 IU



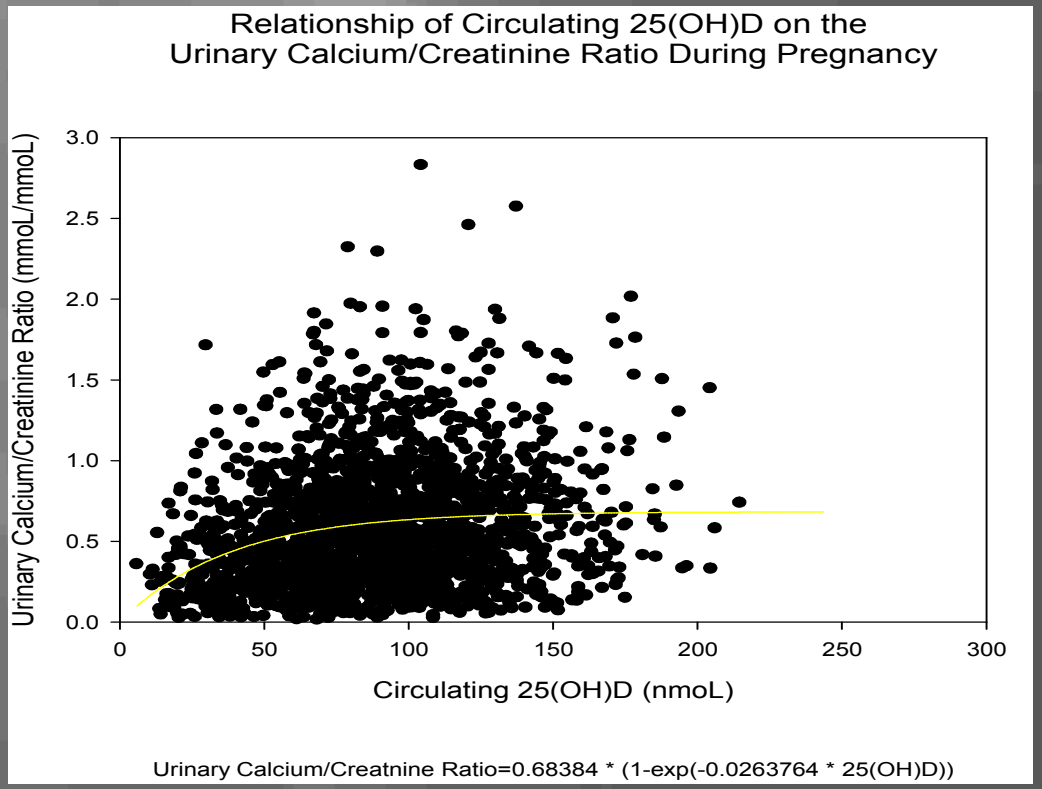
$25(OH)D=97.27*D_3/(0.923+D_3)+0.25*D_3$; $R^2=0.37$; $p<0.0001$


Circulating 1,25(OH)2D (pmol/L) During Pregnancy by Treatment Group
Mean Circulating 1,25(OH)2D (pmol/L) ± S.D.
500
400
300
200
100
0
12
16
20
24
28
32
36
Weeks of Gestation
400 IU
2000 IU
4000 IU



- 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.



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

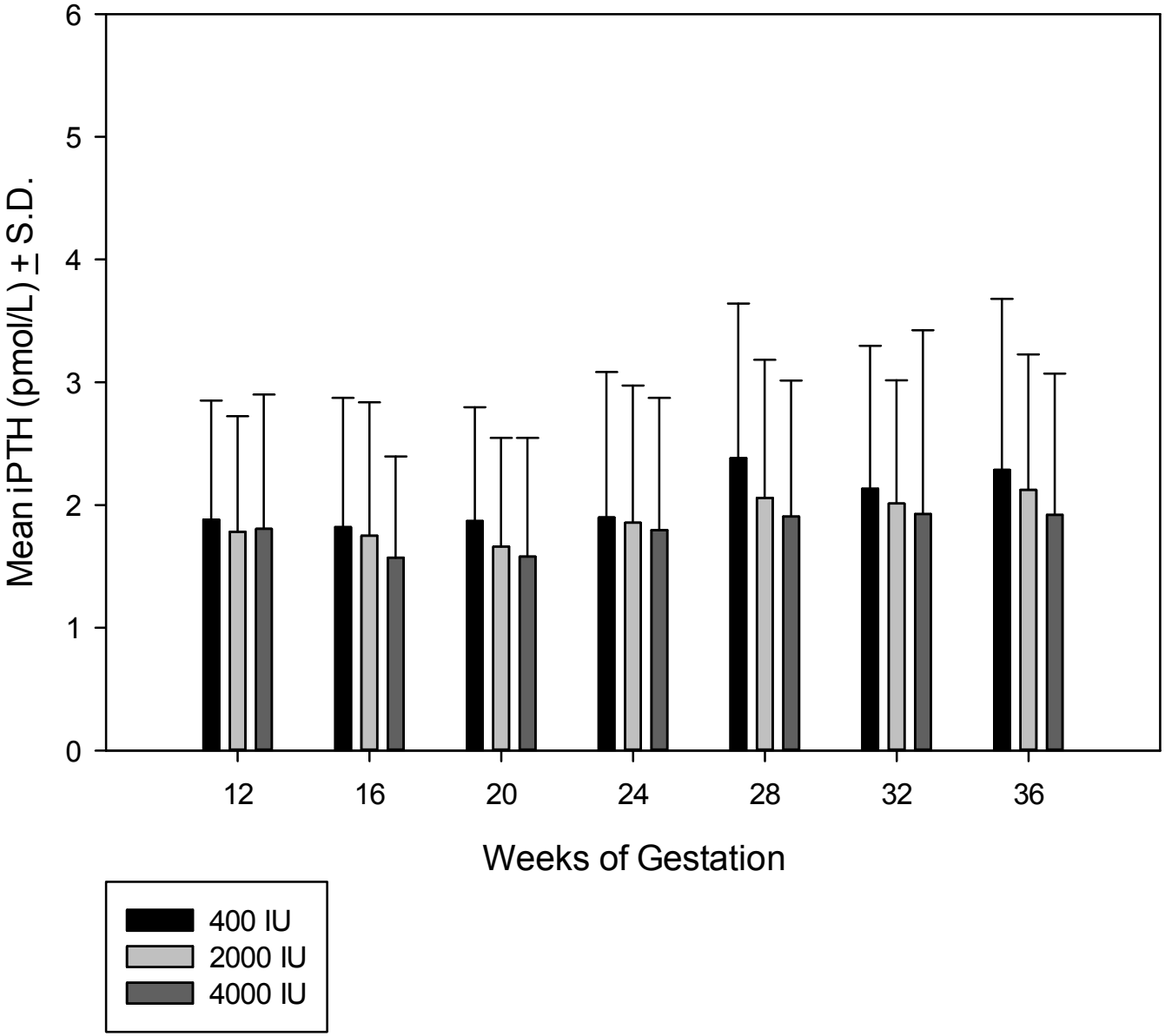


Table 2. Pregnancy Characteristics by Vitamin D Supplementation Group

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	69 (62.2%)	81 (66.4%)	81 (69.8%)		
Assisted vaginal	2 (1.8%)	4 (3.3%)	9 (7.8%)		
C/S after Labor	23 (20.7%)	19 (15.6%)	19 (16.4%)		
C/S without Labor	17 (15.3%)	18 (14.8%)	7 (6.0%)		
Vaginal	71 (74.7%)	85 (79.4%)	90 (85.7%)		
Primary Cesarean Section	24 (25.3%)	22 (20.6%)	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 (%)	47 (42.3)	60 (49.2)	44 (37.6)	0.19	0.4
Bacterial	36 (32.4)	44 (36.1)	32 (27.4)	0.35	0.3
Viral	8 (7.2)	6 (4.9)	6 (5.1)	0.71	0.4
Fungal	13 (11.7)	22 (19.0)	13 (11.1)	0.23	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

Characteristic	Baseline 25(OH)D (means p-value	1 month prior delivery (means p-value)	Delivery (means p-value)	AUC from 20-36 weeks (means p-value)	Mean from 20-36 weeks (means p-value)
Mode of Delivery					
Vaginal	58.6	100.1	96.3	384.3	94.8
Primary Cesarean Section	60.1	103.5	97.4	391.3	98.9
p-value	0.68	0.55	0.86	0.68	0.35
Preterm Birth <37 wks	58.9	83.1	95.7	326.0	83.9
Term Birth	59.5	100.9	100.3	386.2	95.7
p-value	0.91	0.05	0.72	0.04	0.09
Preterm Labor <37 wks in this Pregnancy	52.6	91.4	82.2	357.2	84.0
No PTL	60.7	101.4	98.4	387.1	96.4
p-vlaue	0.03	0.09	0.02	0.11	0.04
Preterm Labor/Preterm Birth <37 wks	54.3	91.1	86.2	357.8	88.6
Uncomplicated	60.7	102.0	98.0	388.5	96.5
p-value	0.057	0.04	0.06*	0.07*	0.058*
Gestational Diabetes	54.8	95.1	84.6	362.0	90.8
No Gestational Diabetes	59.7	100.0	96.4	383.8	95.2
p-value	0.44	0.61	0.32	0.48	0.57
Preeclampsia/Eclampsia/Gest Hyperten	59.5	82.1	80.9	348.5	85.9
No Preeclampsia….	59.5	100.9	96.8	384.7	95.5
p-value	0.99	0.04	0.13	0.22	0.20
Infection (Bacterial, Viral, Fungal)	52.2	27.1	90.6	354.2	87.6
No Infections	65.0	33.2	100.6	404.4	100.7
p-value	<.0001	0.13	0.04	0.0001	<.0001
Bacterial Infections					
None or non-bacterial infections	50.8	89.2	86.0	347.5	85.2
p-value	63.5	104.9	101.2	399.2	99.7
	<.0001	0.0005	0.003	0.0002	<.0001
Co-morbidity (PTB)	55.0	92.9	91.2	360.7	89.5
No Co-morbidity	64.3	107.8	101.5	406.6	101.2
p-value	0.0004	0.0004	0.03	0.0005	0.0004
Co-morbidity (PTL/PTB)	55.3	92.5	90.2	360.2	89.3
No Co-morbidity	65.1	110.3	104.2	412.8	102.8
p-value	0.0003	<.0001	0.005	<.0001	<.0001

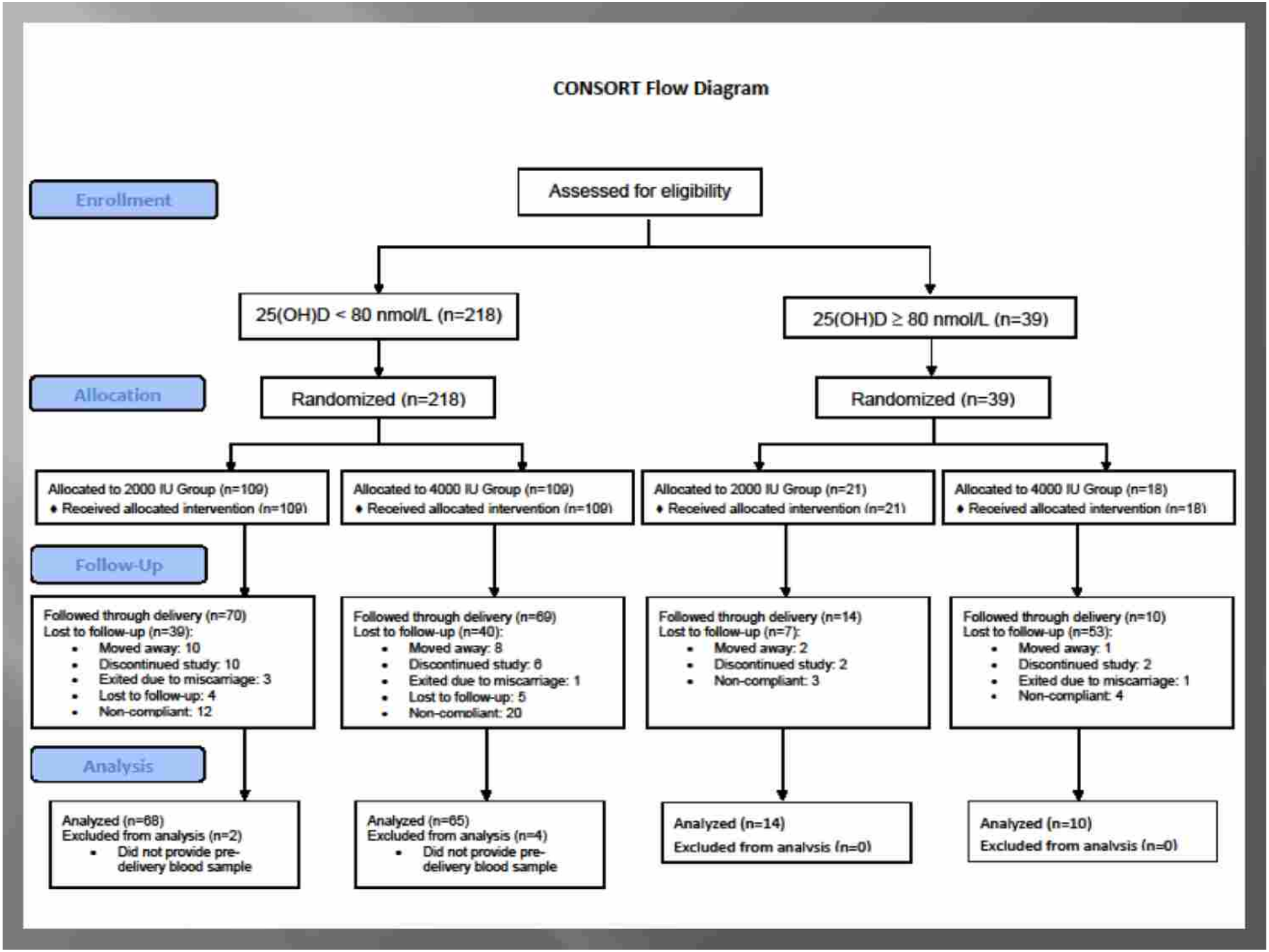
Table 2c. Pregnancy Characteristics by 25(OH)D During Pregnancy Controlling for Ethnicity

Characteristic	Baseline 25(OH)D	1 month prior delivery	Delivery	AUC from 20-36 weeks	Mean from 20-36 weeks
Primary Cesarean Section					
OR	1.003	1.002	1.001	1.001	1.005
95th CI	0.990-1.016	0.994-1.010	0.992-1.010	0.998-1.003	0.995-1.015
p-value	0.67	0.56	0.83	0.67	0.35
Preterm Birth <37 wks					
OR	1.000	0.984	1.000	0.995	0.986
95th CI	0.978-1.021	0.969-0.999	0.982-1.018	0.991-1.000	0.968-1.003
p-value	0.97	0.04	0.97	0.04	0.10
Preterm Labor <37 wks in this Pregnancy					
OR	0.996	0.997	0.992	0.996	0.999
95th CI	0.980-1.012	0.989-1.005	0.982-1.002	0.985-1.006	0.997-1.002
p-value	0.62	0.49	0.10	0.41	0.67
Preterm Labor/Preterm Birth <37 wks					
OR	0.995	0.995	0.994	0.999	0.996
95th CI	0.981-1.010	0.987-1.003	0.985-1.003	0.996-1.001	0.986-1.005
p-value	0.53	0.21	0.18	0.39	0.37
Gestational Diabetes					
OR	0.997	0.999	0.997	0.999	0.998
95th CI	0.972-1.022	0.985-1.013	0.981-1.013	0.995-1.004	0.981-1.016
p-value	0.79	0.84	0.72	0.76	0.85
Preeclampsia/Eclampsia/Gest Hyperten					
OR	1.004	0.985	0.989	0.998	0.989
95th CI	0.981-1.027	0.969-1.00	0.973-1.005	0.993-1.002	0.972-1.007
p-value	0.74	0.05	0.17	0.28	0.23
Infection (Bacterial, Viral, Fungal)					
OR	0.991	0.995	1.000	0.998	0.993
95th CI	0.980-1.002	0.989-1.001	0.992-1.007	0.996-1.000	0.985-1.001
p-value	0.10	0.13	0.91	0.11	0.10
Bacterial Infections					
OR	0.990	0.994	0.995	0.998	0.992
95th CI	0.979-1.002	0.988-1.001	0.988-1.003	0.996-1.000	0.984-1.000
p-value	0.12	0.10	0.21	0.11	0.05
Co-morbidity (PTL/PTB)					
OR	0.994	0.991	0.995	0.997	0.990
95th CI	0.984-1.005	0.984-0.997	0.987-1.002	0.995-1.00	0.982-0.998
p-value	0.29	0.006	0.16	0.02	0.02

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 Vitamin D Pregnancy Clinical Trial


CONSORT Flow Diagram
Enrollment
Assessed for eligibility
25(OH)D < 80 nmol/L (n=218)
25(OH)D ≥ 80 nmol/L (n=39)
Allocation
Randomized (n=218)
Randomized (n=39)
Allocated to 2000 IU Group (n=109)
♦ Received allocated intervention (n=109)
Allocated to 4000 IU Group (n=109)
♦ Received allocated intervention (n=109)
Allocated to 2000 IU Group (n=21)
♦ Received allocated intervention (n=21)
Allocated to 4000 IU Group (n=18)
♦ Received allocated intervention (n=18)
Follow-Up
Followed through delivery (n=70)
Lost to follow-up (n=39):
Moved away: 10
Discontinued study: 10
Exited due to miscarriage: 3
Lost to follow-up: 4
Non-compliant: 12
Followed through delivery (n=69)
Lost to follow-up (n=40):
Moved away: 8
Discontinued study: 6
Exited due to miscarriage: 1
Lost to follow-up: 5
Non-comoliant: 20
Followed through delivery (n=14)
Lost to follow-up (n=7):
Moved away: 2
Discontinued study: 2
Non-compliant: 3
Followed through delivery (n=10)
Lost to follow-up (n=53):
Moved away: 1
Discontinued study: 2
Exited due to miscarriage: 1
Non-compliant: 4
Analysis
Analyzed (n=68)
Excluded from analysis (n=2)
Did not provide pre-delivery blood sample
Analyzed (n=65)
Excluded from analysis (n=4)
Did not provide pre-delivery blood sample
Analyzed (n=14)
Excluded from analysis (n=0)
Analyzed (n=10)
Excluded from analysis (n=0)

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**

Table 1: Sociodemographic and clinical characteristics, by self-reported race/ethnicity

Characteristic	Total Cohort N=257	2000 IU/day N=130	4000 IU/day N=127	p-value	p-value controlled for race
Maternal age (mean ± SD)	25.0 ± 5.1	24.5 ± 5.3	25.4 ± 5.0	0.076	0.103
Gestational age (mean ± SD)	12.4 ± 1.8	12.3 ± 1.7	12.4 ± 2.0	0.34	0.65
Race					
African-American	124 (48.3%)	61 (46.9%)	63 (49.6%)	0.56	--
Caucasian	25 (9.7%)	12 (9.2%)	13 (10.2%)		
Hispanic	101 (39.3%)	55 (42.3%)	46 (36.2%)		
Other	7 (2.7%)	2 (1.5%)	5 (3.9%)		
Highest education achieved					
Less than high school	72 (28.0%)	35 (26.9%)	37 (29.1%)	0.85	0.66
High school	104 (40.5%)	56 (43.1%)	48 (37.8%)		
Some college	57 (22.2%)	27 (20.8%)	30 (23.6%)		
Assoc. degree or higher	24 (9.3%)	12 (9.2%)	12 (9.5%)		
Employed	116 (45.1%)	60 (46.2%)	56 (44.1%)	0.74	0.60
Insurance status					
None	93 (36.2%)	48 (36.9%)	45 (35.4%)	0.96	0.74
Medicaid	47 (18.3%)	23 (17.7%)	24 (18.9%)		
Private	117 (45.5%)	59 (45.4%)	58 (45.7%)		
Subjective health rating	9 (3 - 10)	9 (3 - 10)	10 (5 - 10)	0.29	0.12
Prior obstetrical history					
Preterm birth	12 (4.7%)	5 (3.9%)	7 (5.5%)	0.57	--
Preeclampsia	11 (4.3%)	5 (3.9%)	6 (4.7%)	0.77	--
Gestational diabetes	7 (2.7%)	4 (3.1%)	3 (2.4%)	1.00	--
Diabetes (Type I or II)	5 (2.0%)	2 (1.5%)	3 (2.4%)	0.68	--
Chronic hypertension	2 (0.8%)	0	2 (1.6%)	0.24	--
Planned pregnancy	95 (37.0%)	47 (36.2%)	48 (37.8%)	0.79	0.62
Gravidity	1 (0 - 7)	1 (0 - 7)	1 (0 - 7)	0.37	0.28
Primigravida	97 (37.7%)	51 (39.2%)	46 (36.2%)	0.62	0.55
Parity	1 (0 - 4)	0 (0 - 4)	1 (0 - 4)	0.069	0.067
BMI ≥ 30	70 (27.2%)	32 (24.6%)	38 (29.9%)	0.34	0.32
Season (April - September)	163 (63.4%)	83 (63.9%)	80 (63.0%)	0.89	0.89

Continuous and ordinal variables reported as median (range) unless otherwise noted, and compared between groups using the Wilcoxon rank-sum test. Categorical variables reported as frequency (%), and compared between groups using the chi-square test or Fisher's exact test. P-values controlled for race were obtained using multivariable linear or logistic regression.

Variable	Vitamin D Group	Odds Ratio	95% CI	p-value
Age < 25	<20	1.35	0.82-2.21	0.24
	20-31	1.28	0.77-2.14	0.35
	32+	1.00 (ref)	--	--
Primigravida	<20	0.55	0.26-1.16	0.11
	20-31	0.70	0.31-1.57	0.38
	32+	1.00 (ref)	--	--
Obesity (BMI ≥ 30)	<20	2.19	1.23-3.90	0.008
	20-31	1.21	0.66-2.22	0.54
	32+	1.00 (ref)	--	--
Black (vs. white)	<20	20.28	7.91-52.02	< 0.0001
	20-31	2.96	1.29-6.78	0.007
	32+	1.00 (ref)	--	--
Hispanic (vs. white)	<20	2.44	1.03-5.81	0.046
	20-31	1.44	0.71-2.90	0.51
	32+	1.00 (ref)	--	--
Summer Months (April-September inclusive)	<20	0.60	0.34-1.05	0.073
	20-31	0.88	0.49-1.59	0.67
	32+	1.00 (ref)	--	--

Outcomes

- Overall, mean 25(OH)D change from baseline was 13.6 (SD 13.1) ng/mL, $p<0.001$
  - 12.2 (SD 13.2) ng/mL for the 2000 IU group
  - 15.2 (SD 12.9) ng/mL for the 4000 IU group, $p=0.16$
- In secondary analysis using longitudinal models, the overall rate of increase in 25(OH)D was 2.61 ng/mL per month (95% CI 2.30 - 2.91).
  - The rate of increase differed significantly between the two dose groups ($p=0.035$ for time-dose interaction)
    - 2000 IU group had a rate 0.65/month lower than the 4000 IU group (95% CI for difference in rate -1.26, -0.05)
- Overall, the mean infant 25(OH)D level was 24.5 (SD 12.0) ng/mL;
  - 22.1 (SD 10.3) ng/mL in the 2000 IU group
  - 27.0 (SD 13.3) ng/mL in the 4000 IU group ($p=0.024$)

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.

Another look…

Combined NICHD and Thrasher Cohorts

Combined NICHD and Thrasher Cohorts

Site	400 IU Group N=111	2000 IU Group N=205	4000 IU Group N=197
Thrasher	0 (0.0)	83 (40.5)	80 (40.6)
MUSC	111 (100.0)	122 (59.5)	117 (59.4)

Sorting by race/ethnicity…

	African American			Hispanic			Caucasian		
Mean 25(OH)D	Baseline	2nd Trimester	Before Delivery	Baseline	2nd Trimester	Before Delivery	Baseline	2nd Trimester	Before Delivery
Preterm Birth <37 wks	38.0	57.7	63.1	55.6	89.3	78.0	75.8	101.7	104.7
Term	43.2	69.2	83.1	61.5	87.6	102.4	74.1	100.0	115.1
Difference	5.2	11.4	19.9	5.9	-1.7	24.4	-1.7	-1.7	10.3
Preterm Labor/Birth <37 wks	40.1	63.3	75.5	56.5	88.8	85.6	75.0	99.9	112.7
Term	44.3	71.0	84.7	61.6	87.6	102.1	74.0	100.2	114.8
Difference	4.2	7.7	8.2	5.1	-1.1	16.5	-0.99	0.3	2.1

Although not statistically significant, there is a clear trend among women of similar race: lower mean 25(OH)D in women who deliver <37 wks and/or who experienced premature labor.

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

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

Thank You