HB

90

<TARGET><BILL>HB 90</BILL><SUBJECT>HB
90</SUBJECT><COMM>HHSS28</COMM></TARGET>

HOUSE COMMITTEE REPOI

(7)
Date Referred to Committee: January 30, 2013 **FURTHER REFERRALS: Finance**

Date of Committee Action: 4/8/2014

The HEALTH AND SOCIAL SERVICES Committee considered: **HB 90**

HOUSE BILL NO. 90
"An Act establishing a temporary program in the Department of Health and Social Services for testing newborns for baseline vitamin D levels."

HB 90 VITAMIN D SUPPLEMENTS

Recommends it be replaced with [ ] HCS *or* [X] CS *for* HB 90 (HSS)
For Senate Bills with new title: [ ] Technical Title [ ] New Title: HCR_____ **[ ] Same Title [X] New Title**

[ ] attach amendments
[ ] add new referral to _____Committee
[ ] Letter of Intent_____Committee

List of Abbrev for Depts.:
ADM
CED
COR
CRT
EED
DEC
DFG
GOV
DHS
LWF
LAW
LEG
MVA
DNR
DPS
REV
DOT
UA

***NEW* FISCAL NOTES**
*FN# is assigned by Chief Clerk's Office

*FN#	List by Dept(s):	Fiscal	Indet.	Zero
	DHS	✓		

***PREVIOUS* FISCAL NOTES**

FN#	List by Dept(s):	Fiscal	Indet.	Zero

Signing with recommendations	Printed Last Name	DP	DNP	NR	AM
Paul Seaton	SEATON	X			
Sue Reinbold	Reinbold			X	
Lee Keller	Pettitt			X	
Wes Keller	Keller			X	
Bernard	Tarr	X			
Chair: Allen	Higgins	✓			
Chair:					

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REPRESENTATIVE Paul Seaton
District 30

HB 90
Explanation of Changes: Version O to Version Y

The changes in Committee Substitute for House Bill 90 work draft 28-LS0376\Y are intended to shift the legislation from a temporary *research* project to a temporary *health* project involving both mothers and newborns.

Changes

- 'Research' has been removed from the title and replaced with 'health.' The title change also includes mothers in the project and adds supplementation and nutritional education.

- In **subsection (a)**, the legislative findings have been expanded to include the reduction of negative pregnancy outcomes with adequate vitamin D.

- In **subsection (b)** the project length has been updated from 12 months to 24 months, 'research' has been removed from the project description, and the voluntary project population of 500 pregnant women and their newborns has replaced the previous testing group of all Alaskan newborns.

- **Subsection (c)** describes a collaboration between the department and an interested nonprofit entity to provide the services under the health project, and limits the cost to the department to the lesser of $100,000 or 20% of the project cost. The services described in this subsection include items relating to blood testing, which were listed under subsection (d) of the previous draft. It also outlines the three main goals of the temporary health project: determining vitamin D levels of the participants, providing vitamin D supplementation as needed, and providing information to the participants and the public on the project and on vitamin D sufficiency.

- **Subsection (d)** directs the department to contract with a qualified research group to provide technical support as well as analysis for the samples and data collected during this health project. Subsection (d) is similar to the first paragraph of subsection (c) of the previous draft.

- **Subsection (e)** and **subsection (f)** have been updated to ensure the language conforms with other sections that have been changes, but remain substantially similar.

28-LS0376\Y
Mischel
3/31/14

CS FOR HOUSE BILL NO. 90( )

IN THE LEGISLATURE OF THE STATE OF ALASKA

TWENTY-EIGHTH LEGISLATURE - SECOND SESSION

BY

Offered:
Referred:

Sponsor(s): REPRESENTATIVES SEATON, Gruenberg

A BILL

FOR AN ACT ENTITLED

"An Act relating to a temporary health project for testing mothers and newborns for baseline vitamin D levels and supplying supplementation and nutritional education."

BE IT ENACTED BY THE LEGISLATURE OF THE STATE OF ALASKA:

*** Section 1.** The uncodified law of the State of Alaska is amended by adding a new section to read:

VITAMIN D TESTING FOR PREGNANT WOMEN AND NEWBORNS. (a) The legislature finds that multiple studies demonstrate a link between vitamin D insufficiency in newborns and higher incidences of mental and physical health problems that lead to higher costs of future medical, educational, and support services. Studies also show lower rates of negative pregnancy outcomes with adequate vitamin D levels.

(b) On or before January 1, 2015, the department shall coordinate a 24-month project for the purpose of acquiring data on vitamin D levels of 500 pregnant women and their newborns born in the state during the testing period as provided in this section.

(c) The department shall award a matching grant of not more than 20 percent of the

entire project cost, not to exceed $100,000, to a nonprofit entity to provide, at no cost to the health care provider or to the participant, the following services:

(1) initiate a voluntary health project in the state to

(A) determine vitamin D levels of pregnant women and their newborns;

(B) provide vitamin D supplementation, as needed, to project participants; and

(C) provide public information regarding the project and the effects of vitamin D deficiency;

(2) take or supervise the taking of blood-spot samples from approximately 500 volunteer pregnant women and their newborns for vitamin D level testing;

(3) conduct, to the extent feasible, the vitamin D testing at the same time as other newborn or cord blood testing;

(4) ensure that the sampling complies with federal and state privacy laws;

(5) submit the samples to the group that is under contract with the department to analyze the samples; and

(6) provide follow-up to the project participants as necessary to provide nutritional education and vitamin D supplementation, if warranted.

(d) The department shall contract with a research group that is affiliated with an accredited university in the United States and that is conducting national clinical research on the subject of newborn and prenatal vitamin D levels to provide technical support and to analyze the samples and data collected under (c) of this section.

(e) The department shall notify health care providers licensed in the state of the existence of the project and provide contact information for the grantee under (c) of this section.

(f) In this section,

(1) "department" means the Department of Health and Social Services;

(2) "health care provider" means a physician, physician assistant, midwife, or nurse.

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REPRESENTATIVE Paul Seaton
District 30

HB 90- Vitamin D Supplements
Sponsor Statement

Recent pediatric studies relating to vitamin D levels and infant development have added to growing evidence of the importance of vitamin D sufficiency in areas such as language, psychomotor, and mental development, demonstrating an association between low vitamin D levels in newborns and higher risks of mental and physical health problems. Furthermore they connect vitamin D deficiency to several factors relevant to Alaska such as northern latitude, skin pigmentation, and covering. Additional research has revealed that pregnant women with adequate vitamin D levels experience a lower rate of negative pregnancy outcomes.

HB 90 is a temporary law establishing a two year health project to provide vitamin D testing, health information, and necessary supplementation for approximately 500 participating pregnant Alaskan women and their newborns. This project would give us data on the baseline vitamin D levels of these Alaskans while helping them avoid negative pregnancy outcomes and infant development issues. For instance, research in South Carolina by Dr. Wagner et al. has demonstrated that supplementing to sufficient levels of vitamin D during pregnancy can reduce the number of pre-term births to 7%, more than 2% *below* the March of Dimes 2020 goal of 9.6%.

The positive implications of HB 90 are not just improved health, but also economic savings. One preterm birth costs an average of $55,000; in the proposed project group of 500 Alaskan women, a rate of reduction of preterm births similar to South Carolina would mean in savings of $1,375,000.

Sufficient vitamin D is important to Alaskans' health and savings, but currently we only have a limited idea of what our levels are. This temporary health project will provide baseline data on a portion of our population while improving their health outcomes, and will be an important first step in establishing the health of the greater Alaskan population.

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REPRESENTATIVE Paul Seaton
District 30

Memorandum

To: Representative Pete Higgins, Chairman- Health and Social Services Committee

From: Representative Paul Seaton

Date: February 4, 2014

RE: Explanation of Changes: Proposed CS HB 90 Version O

Explanation of Changes: Proposed CS Version O for HB 90

The Committee Substitute for House Bill 90, work draft 28-LS0376\O makes the following changes.

Language throughout the bill has been reordered and in some cases changed to indicate importance and to reflect the purpose of the legislation, which is to establish a baseline vitamin D level for Alaska through a temporary contract with a qualified and knowledgeable group.

The specific changes are as follows:

- The title has been altered to reflect changes made within the bill and to indicate a more specific focus for the legislation.
- In **subsection (b),** language has been deleted and modified. The purpose of this subsection is to initiate the contract for a temporary vitamin D research project. The changes are as follows:
    - The effective start date has been changed from January 1, 2014 to January 1, 2015.
    - The language has been changed to clarify that the department is to initiate a contract for a yearlong testing project. Previous language had placed responsibility for a program within the department.

- **Subsection (c)** includes language which clarifies the type of research group the department should contract with for this project and the requirements of the contract. The purpose of this subsection is to define the type of contract the department is to enter into for this project.
  The requirements are as follows:

  - A limit of not more than $60 for the testing and analytical work of each sample, which maintains the cost at the lowest know rate for this service.

  - A requirement that the test results be returned to provider who conducted or oversaw the test taking.

  - A requirement to report the test results to the department without identifying the newborn, allowing the state to map the vitamin D level of the newborn across the state while maintaining privacy.

  - Allowing the cooperation between the contractor, provider and department for distribution of samples and reporting of data.

  - Other provisions necessary to establish the contract.

- **Subsection (d)** includes language describing the role of the attending certified health provider in conducting or supervising the test, as well as ensuring compliance with the testing program and any federal or state privacy laws. Subsection B also states that these provisions shall be undertaken at no cost to the provider or the parent of the newborn and allows for parents to claim religious exemption from the testing. Similar language was included in the previous Committee Substitute. This version expands the guarantee of no cost to the provider.
  This subsection clarifies the duties of the health care provider, ensures that the samples are submitted to the proper testing group, ensures that testing under this project will be at no cost to the parent of the newborn or the provider, and makes clear the privacy and exemption rights of the parent and newborn.
  The provider responsibilities are as follow:

  - The provider is responsible for taking or supervising the taking of blood-spot testing from the cord blood of each newborn for the purpose of vitamin D testing.

  - The testing shall be conducted at the same time as other newborn blood tests already required by the state.

- The sampling done under a certified provider must comply with federal and state privacy laws.
- The provider who takes or supervises the sample is responsible for returning the samples to the contracted research group.
- The provider will conduct a follow-up with the parent of a newborn if such a follow up is within the provider's standards of practice.

- **Subsection (e)** is a new section in House Bill 90 which instructs the department to notify health care providers in the state of the existence of the program , the terms of the contract, and the contact information for the contracted research group.
  This section is meant to ensure the communication between providers, the department, and the contractor necessary to effectively pursue this project.

- **Subsection (f)** defines terms used in the bill.

28-LS0376\O
Mischel
2/4/14

CS FOR HOUSE BILL NO. 90( )

IN THE LEGISLATURE OF THE STATE OF ALASKA

TWENTY-EIGHTH LEGISLATURE - SECOND SESSION

BY

Offered:
Referred:

Sponsor(s): REPRESENTATIVES SEATON, Gruenberg

A BILL

FOR AN ACT ENTITLED

"An Act relating to a temporary research project for testing newborns for baseline vitamin D levels."

BE IT ENACTED BY THE LEGISLATURE OF THE STATE OF ALASKA:

*** Section 1.** The uncodified law of the State of Alaska is amended by adding a new section to read:

NEWBORN TESTING PROGRAM FOR VITAMIN D; LEGISLATIVE FINDINGS. (a) The legislature finds that multiple studies demonstrate a link between vitamin D insufficiency in newborns and higher incidences of mental and physical health problems that lead to higher costs of future medical, educational, and support services.

(b) On or before January 1, 2015, the department shall initiate and coordinate a 12-month research project for the purpose of acquiring baseline data on vitamin D levels of newborns born in the state during the testing period as provided in this section.

(c) The department shall contract with a research group that is affiliated with an accredited university in the United States and that is conducting national clinical research on

the subject of newborn and prenatal vitamin D levels to provide laboratory and analytic services for the samples collected under this section. The contract must provide for

(1) a cost of not more than $60 for testing and analytical work of each sample;

(2) reporting of test results to the provider who took the sample;

(3) reporting of test results to the department without identifying information of the newborn;

(4) cooperation among the contractor, the provider, and the department for provision of the samples and reporting of data; and

(5) other provisions the department considers necessary.

(d) A health care provider licensed in the state who attends the delivery of a newborn during the term of the contract under (c) of this section shall, at no cost to the provider or to the parent of the newborn, unless the parent of a newborn refuses testing based on a religious tenet,

(1) take or supervise the taking of blood-spot samples from each newborn or the cord blood for vitamin D level testing;

(2) conduct, to the extent feasible, the vitamin D testing at the same time as other newborn or cord blood testing;

(3) ensure the sampling complies with federal and state privacy laws;

(4) submit the samples to the group that is under contract with the department to analyze the samples; and

(5) provide follow-up to the parent of the newborn as necessary to meet the standards of practice of the provider.

(e) The department shall notify health care providers licensed in the state of the existence of the research project initiated under this section, contact information for the contractor under (c) of this section, and the terms of the contract.

(f) In this section,

(1) "department" means the Department of Health and Social Services;

(2) "health care professional" means a physician, physician assistant, midwife, or nurse.

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REPRESENTATIVE Paul Seaton
District 30

HB 90- Vitamin D Supplements
Sponsor Statement

Recent pediatric studies relating to vitamin D levels and infant development have added to growing evidence of the importance of vitamin D sufficiency in areas such as language, psychomotor, and mental development. These studies demonstrate an association between low vitamin D levels in newborns and higher risks of mental and physical health problems. The studies also connect vitamin D deficiency to several factors relevant to Alaska. Northern latitude, skin pigmentation, and Alaskan's predominantly long sleeve clothing may all lead to low levels in our residents. **HB 90** is a temporary law, establishing a year-long project to test Alaska's newborn vitamin D levels. The samples collected during the HB 90 testing period will be sent to an accredited laboratory to provide testing and analytical services. By testing across the wide spectrum of locations and population groups, we will gain insight into how our population is affected by Alaska's northern latitude and which subgroups are at a greater risk of vitamin D deficiency. Understanding and targeting those at-risk Alaskans revealed by the study at the newborn and neonatal level, when important brain development is occurring, could lead to future savings in medical, educational, and support service costs. Depending on the Alaskan Newborn results, demonstrated links between neural development and vitamin D levels may point to vitamin D deficiency as a limiting factor in reaching our State's educational goals.

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REPRESENTATIVE Paul Seaton
District 30

MEMORANDUM

TO: Representative Pete Higgins, Chairman- Health and Social Services Committee

FROM: Representative Paul Seaton

DATE: February 15, 2013

RE: Explanation of Changes in CS HB 90

Explanation of Changes in CS HB 90

The Committee Substitute for House Bill 90, work draft 28-LS0376/U makes the following changes:

In section 1, subsection d, line 10 the word 'laboratory' is deleted.
In section 1, subsection d, line 10 the deleted text is replaced with the phrase 'research group.'

We requested that these changes be made because we felt the initial language was too specific, and might prevent the department from contracting with an otherwise qualified and capable institution.

28-LS0376\U
Mischel
2/14/13

CS FOR HOUSE BILL NO. 90( )

IN THE LEGISLATURE OF THE STATE OF ALASKA

TWENTY-EIGHTH LEGISLATURE - FIRST SESSION

BY

Offered:
Referred:

Sponsor(s): REPRESENTATIVES SEATON, Reinbold, Gruenberg

A BILL

FOR AN ACT ENTITLED

"An Act establishing a temporary program in the Department of Health and Social Services for testing newborns for baseline vitamin D levels."

BE IT ENACTED BY THE LEGISLATURE OF THE STATE OF ALASKA:

*** Section 1.** The uncodified law of the State of Alaska is amended by adding a new section to read:

NEWBORN TESTING PROGRAM FOR VITAMIN D; LEGISLATIVE FINDINGS. (a) The legislature finds that multiple studies demonstrate a link between vitamin D insufficiency in newborns and higher incidences of mental and physical health problems that lead to higher costs of future medical, educational, and support services.

(b) Beginning on or before January 1, 2014, the Department of Health and Social Services shall establish a 12-month statewide program for testing the vitamin D levels of newborns at birth or as soon after birth as possible for the purpose of acquiring baseline vitamin D levels of all newborns in the state during the testing period. The testing shall be conducted, at no cost to a parent or guardian of the newborn, by or under the supervision of a

health care professional licensed in the state who attends the delivery.

(c) The program established under this section must include a procedure for

(1) combining, to the extent feasible, the vitamin D testing with other newborn or cord blood testing;

(2) ensuring testing complies with federal and state privacy laws;

(3) reporting test results to the department and to the parent or guardian of the newborn; and

(4) permitting a mother of a newborn to refuse testing if serologic testing is contrary to the tenets or practice of the religious creed of the mother.

(d) The department shall contract with a research group that is affiliated with an accredited university in the United States and that is conducting national clinical research on the subject of newborn and prenatal vitamin D levels to provide laboratory and analytic services for the samples collected under this section.

(e) In this section, "health care professional" means a physician, physician assistant, midwife, or nurse.

CS FOR HOUSE BILL NO. 90(HSS)

IN THE LEGISLATURE OF THE STATE OF ALASKA

TWENTY-EIGHTH LEGISLATURE - SECOND SESSION

BY THE HOUSE HEALTH AND SOCIAL SERVICES COMMITTEE

Offered:
Referred:

Sponsor(s): REPRESENTATIVES SEATON, Gruenberg

A BILL

FOR AN ACT ENTITLED

"An Act relating to a temporary health project for testing mothers and newborns for baseline vitamin D levels and supplying supplementation and nutritional education."

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(b) On or before January 1, 2015, the department shall coordinate a 24-month project for the purpose of acquiring data on vitamin D levels of 500 pregnant women and their newborns born in the state during the testing period as provided in this section.

(c) The department shall award a matching grant of not more than 20 percent of the

entire project cost, not to exceed $100,000, to a nonprofit entity to provide, at no cost to the health care provider or to the participant, the following services:

(1) initiate a voluntary health project in the state to

(A) determine vitamin D levels of pregnant women and their newborns;

(B) provide vitamin D supplementation, as needed, to project participants; and

(C) provide public information regarding the project and the effects of vitamin D deficiency;

(2) take or supervise the taking of blood-spot samples from approximately 500 volunteer pregnant women and their newborns for vitamin D level testing;

(3) conduct, to the extent feasible, the vitamin D testing at the same time as other newborn or cord blood testing;

(4) ensure that the sampling complies with federal and state privacy laws;

(5) submit the samples to the group that is under contract with the department to analyze the samples; and

(6) provide follow-up to the project participants as necessary to provide nutritional education and vitamin D supplementation, if warranted.

(d) The department shall contract with a research group that is affiliated with an accredited university in the United States and that is conducting national clinical research on the subject of newborn and prenatal vitamin D levels to provide technical support and to analyze the samples and data collected under (c) of this section.

(e) The department shall notify health care providers licensed in the state of the existence of the project and provide contact information for the grantee under (c) of this section.

(f) In this section,

(1) "department" means the Department of Health and Social Services;

(2) "health care provider" means a physician, physician assistant, midwife, or nurse.

IMPLICATIONS OF LOW VITAMIN D FOR ALASKAN CHILDREN

House Bill 90

Representative Paul Seaton

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Vitamin D: Implications for Alaskan Children

Representative Paul Seaton

Emphasis marks and distributed by Representative Seaton

PEDIATRICS®
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Australia

Language Impairment

Maternal Serum Vitamin D Levels During Pregnancy and Offspring Neurocognitive Development

Andrew J. O. Whitehouse, Barbara J. Holt, Michael Serralha, Patrick G. Holt, Merci M. H. Kusel and Prue H. Hart

Pediatrics 2012;129;485; originally published online February 13, 2012;
DOI: 10.1542/peds.2011-2644

Maternal Serum Vitamin D Levels During Pregnancy and Offspring Neurocognitive Development

AUTHORS: Andrew J. O. Whitehouse, PhD, Barbara J. Holt, BSc, Michael Serralha, BSc(Hons), Patrick G. Holt, DSc, Merci M. H. Kusel, MBBS, and Prue H. Hart, PhD

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KEY WORDS
vitamin D, neurocognitive, language impairment, behavioral problems, emotional problems, Raine study

ABBREVIATIONS
95% CI—95% confidence interval
CBCL—Child Behavior Checklist
OR—odds ratio
PPVT-R—Peabody Picture Vocabulary Test—Revised

Ms Kusel and Dr Hart contributed equally to this work.

Drs Whitehouse, Kusel, and Hart developed the hypotheses; Ms Holt, Mr Serralha, Dr Holt, and Dr Hart analyzed serum samples for 25(OH)-vitamin D concentrations; and Dr Whitehouse conducted the statistical analyses and wrote the main drafts of the manuscript. All authors contributed to the interpretation and discussion of the results and other sections of the manuscript.

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FINANCIAL DISCLOSURE: *The authors have indicated they have no financial relationships relevant to this article to disclose.*

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WHAT'S KNOWN ON THIS SUBJECT: Vitamin D levels in the general population have decreased considerably over the past decade. The implications of maternal vitamin D insufficiency during pregnancy for offspring neurocognitive development remain unclear.

WHAT THIS STUDY ADDS: Studying a large sample and using a prospective longitudinal design, this study demonstrates a link between maternal vitamin D insufficiency during pregnancy and offspring language impairment. There was no association with childhood behavioral or emotional problems.

abstract

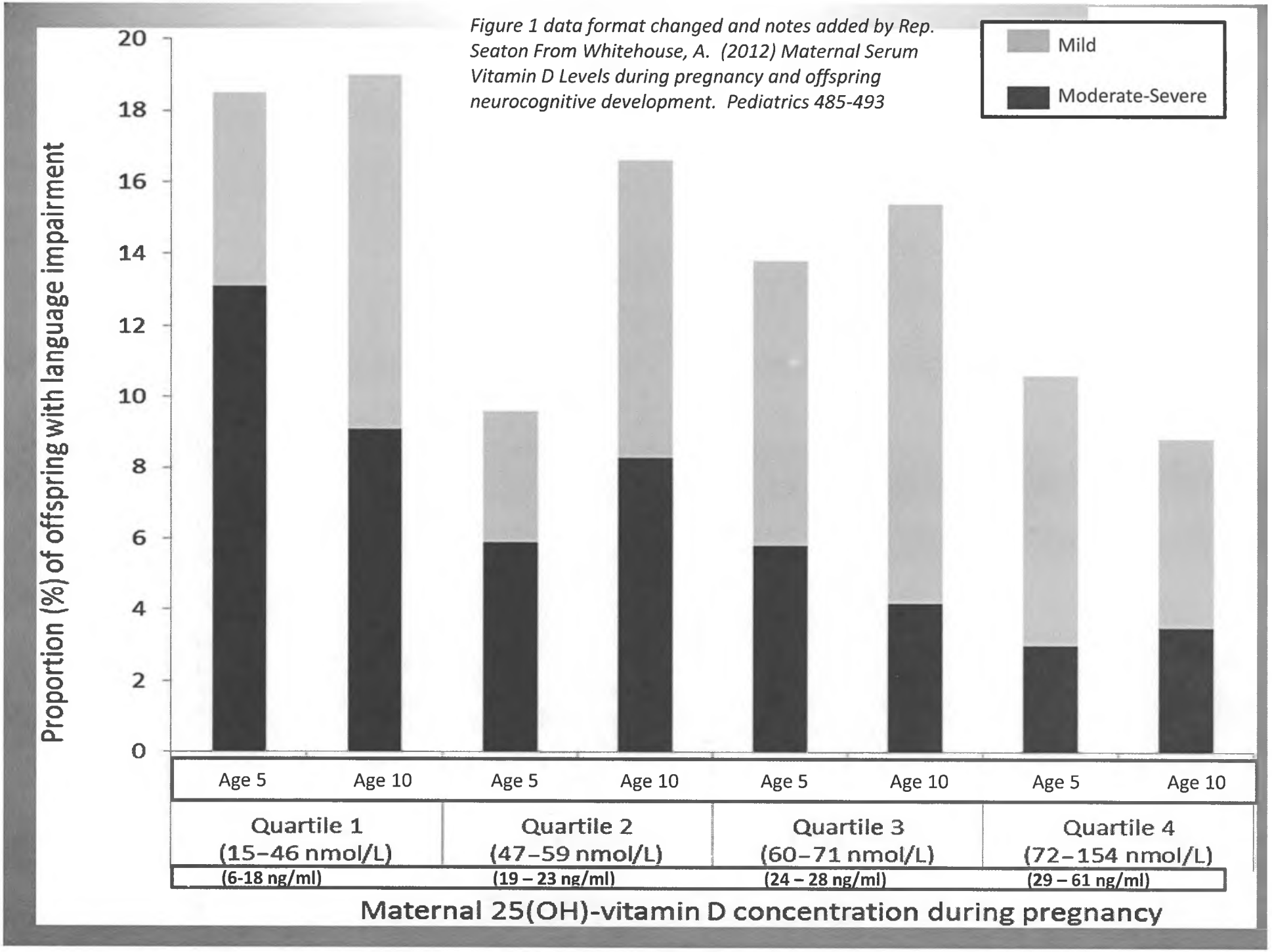
OBJECTIVE: To determine the association between maternal serum 25(OH)-vitamin D concentrations during a critical window of fetal neurodevelopment and behavioral, emotional, and language outcomes of offspring.

METHODS: Serum 25(OH)-vitamin D concentrations of 743 Caucasian women in Perth, Western Australia (32°S) were measured at 18 weeks pregnancy and grouped into quartiles. Offspring behavior was measured with the Child Behavior Checklist at 2, 5, 8, 10, 14, and 17 years of age (n range = 412–652). Receptive language was assessed with the Peabody Picture Vocabulary Test—Revised at ages 5 (n = 534) and 10 (n = 474) years. Raw scores were converted to standardized scores, incorporating cutoffs for clinically significant levels of difficulty.

RESULTS: χ^2 analyses revealed no significant associations between maternal 25(OH)-vitamin D serum quartiles and offspring behavioral/emotional problems at any age. In contrast, there were significant linear trends between quartiles of maternal vitamin D levels and language impairment at 5 and 10 years of age. Multivariate regression analyses, incorporating a range of confounding variables, found that the risk of women with vitamin D insufficiency (≤46 nmol/L) during pregnancy having a child with clinically significant language difficulties was increased close to twofold compared with women with vitamin D levels >70 nmol/L.

CONCLUSIONS: Maternal vitamin D insufficiency during pregnancy is significantly associated with offspring language impairment. Maternal vitamin D supplementation during pregnancy may reduce the risk of developmental language difficulties among their children. *Pediatrics* 2012;129:485–493

Figure 1 data format changed and notes added by Rep. Seaton From Whitehouse, A. (2012) Maternal Serum Vitamin D Levels during pregnancy and offspring neurocognitive development. Pediatrics 485-493



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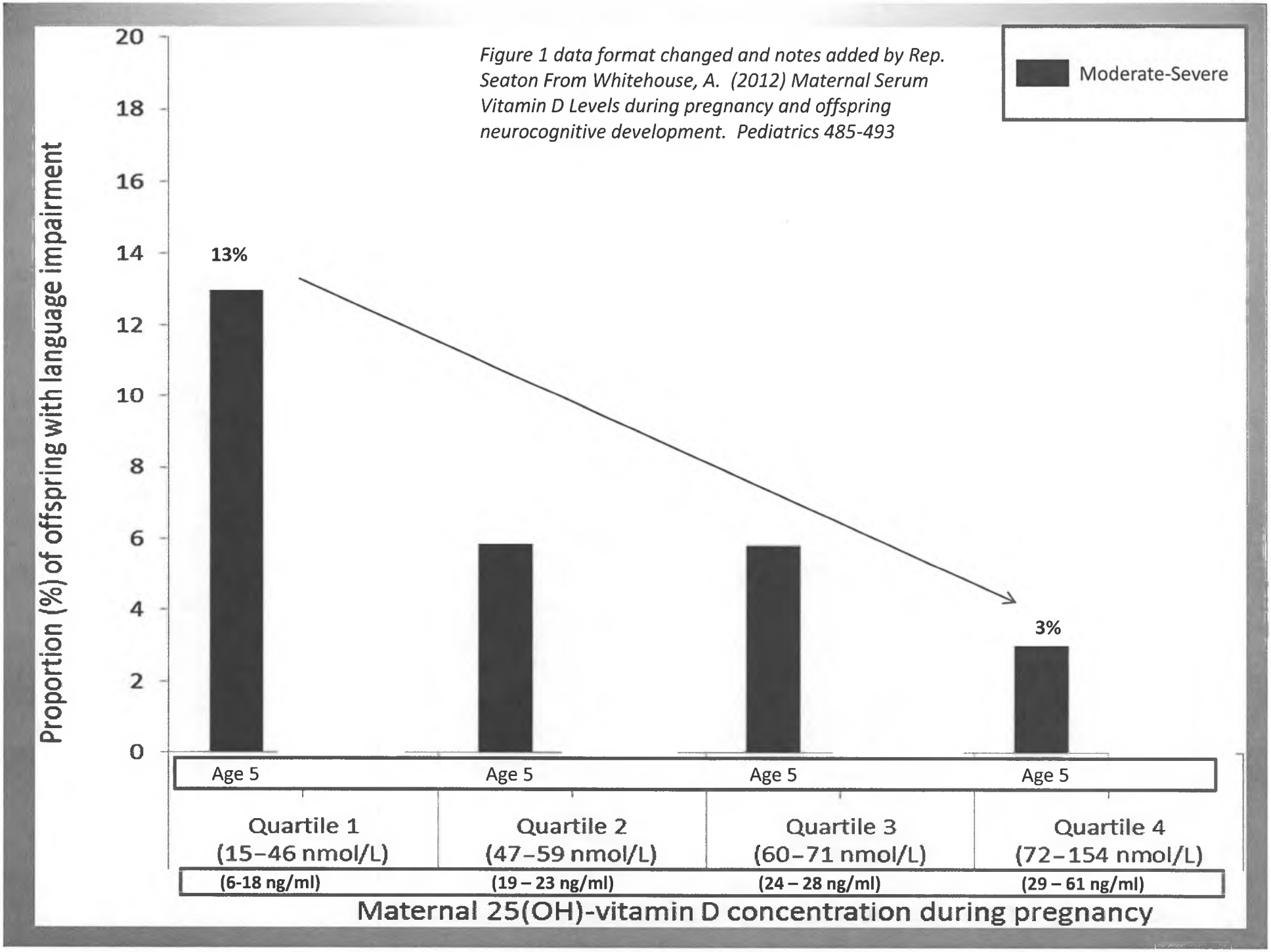

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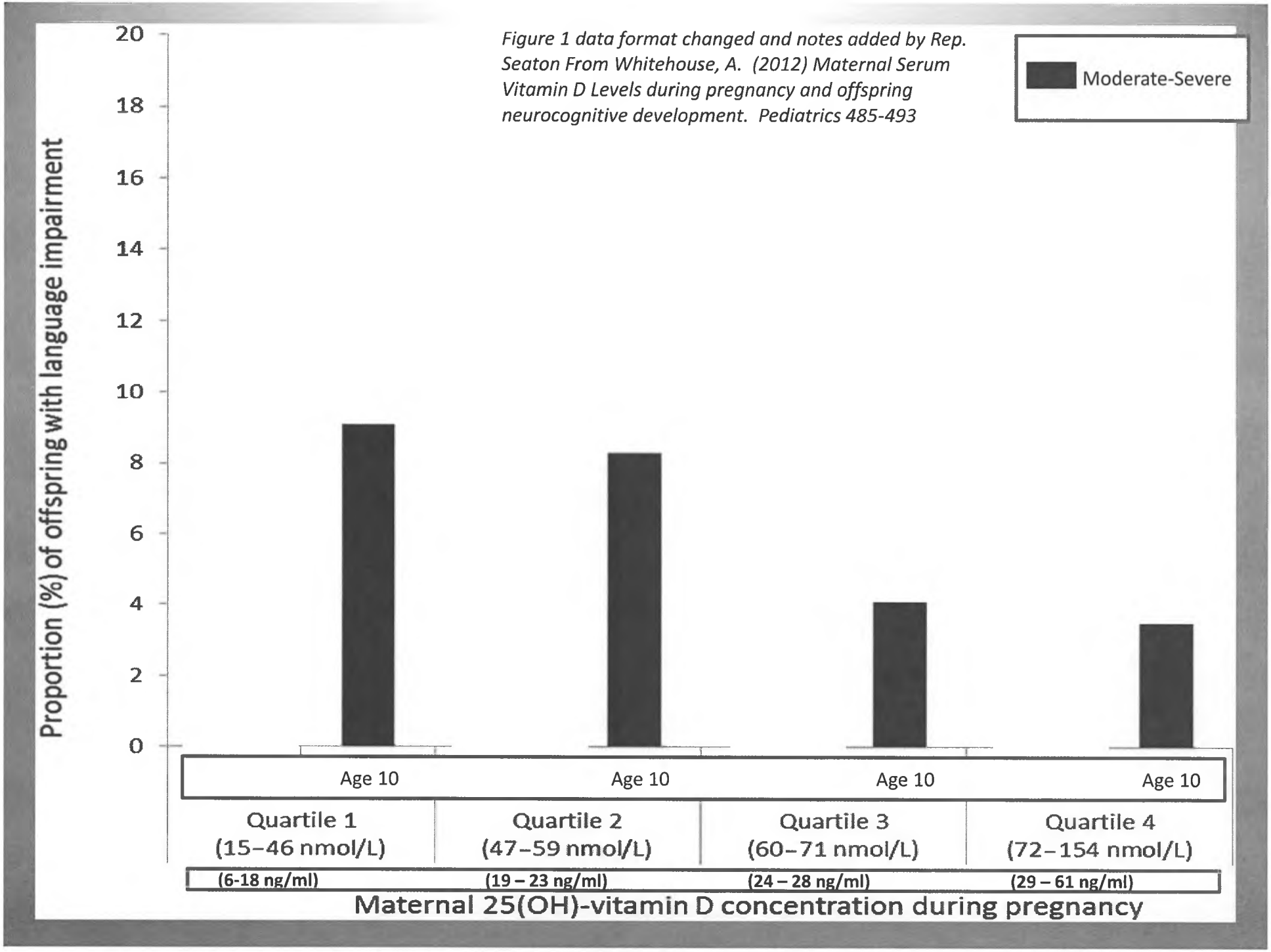

Emphasis marks and distributed by Representative Seaton

PEDIATRICS®
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Spain

Mental and Psychomotor Development

Circulating 25-Hydroxyvitamin D_3 in Pregnancy and Infant Neuropsychological Development

Eva Morales, Mònica Guxens, Sabrina Llop, Clara L. Rodríguez-Bernal, Adonina Tardón, Isolina Riaño, Jesús Ibarluzea, Nerea Lertxundi, Mercedes Espada, Agueda Rodriguez and Jordi Sunyer

Pediatrics 2012;130;e913; originally published online September 17, 2012;
DOI: 10.1542/peds.2011-3289

Circulating 25-Hydroxyvitamin D_3 in Pregnancy and Infant Neuropsychological Development

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KEY WORDS

child development, cognition, infancy, intelligence, vitamin D

ABBREVIATIONS

CI—95% confidence intervals
FP—fractional polynomial
$25(OH)D_3$—25-hydroxyvitamin D_3

*Drs Morales and Guxens contributed equally to this work.

Dr Sunyer had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; Drs Morales, Guxens, Sunyer were responsible for study concept and design; Drs Guxens, Llop, Tardón, Ibarluzea, Espada, and Sunyer were responsible for acquisition of data; Drs Morales and Guxens were responsible for drafting of the manuscript; Drs Llop, Rodríguez-Bernal, Tardón, Riaño, Ibarluzea, Lertxundi, Espada, Rodríguez, and Sunyer were responsible for critical revision of the manuscript for important intellectual content; Dr Guxens was responsible for statistical analysis; and Drs Ibarluzea, Tardón, and Sunyer obtained funding.

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(Continued on last page)

WHAT'S KNOWN ON THIS SUBJECT: Adequate vitamin D status in mothers during pregnancy may influence the health status of offspring later in life. Growing evidence based on animal studies is linking vitamin D to brain development and functioning, but studies in humans are lacking.

WHAT THIS STUDY ADDS: This large-scale prospective pregnancy cohort study examines the association between maternal circulating 25-hydroxyvitamin D_3 concentrations in pregnancy and offspring neuropsychological development. Higher circulating concentration of 25-hydroxyvitamin D_3 in pregnancy was associated with improved mental and psychomotor development in infants.

abstract

OBJECTIVE: To investigate whether circulating 25-hydroxyvitamin D_3 [$25(OH)D_3$] concentration in pregnancy is associated with neuropsychological development in infants.

METHODS: The Spanish population-based cohort study INfancia y Medio Ambiente Project recruited pregnant women during the first trimester of pregnancy between November 2003 and February 2008. Completed data on 1820 mother-infant pairs were used. Maternal plasma $25(OH)D_3$ concentration was measured by high-performance liquid chromatography in pregnancy (mean 13.5±2.1 weeks of gestation). Offspring mental and psychomotor scores were assessed by trained psychologists at age 14 months (range, 11–23) by using the Bayley Scales of Infant Development. β-Coefficients with 95% confidence intervals (CIs) of mental and psychomotor scores associated with continuous or categorical concentrations of maternal plasma $25(OH)D_3$ were calculated by using linear regression analysis.

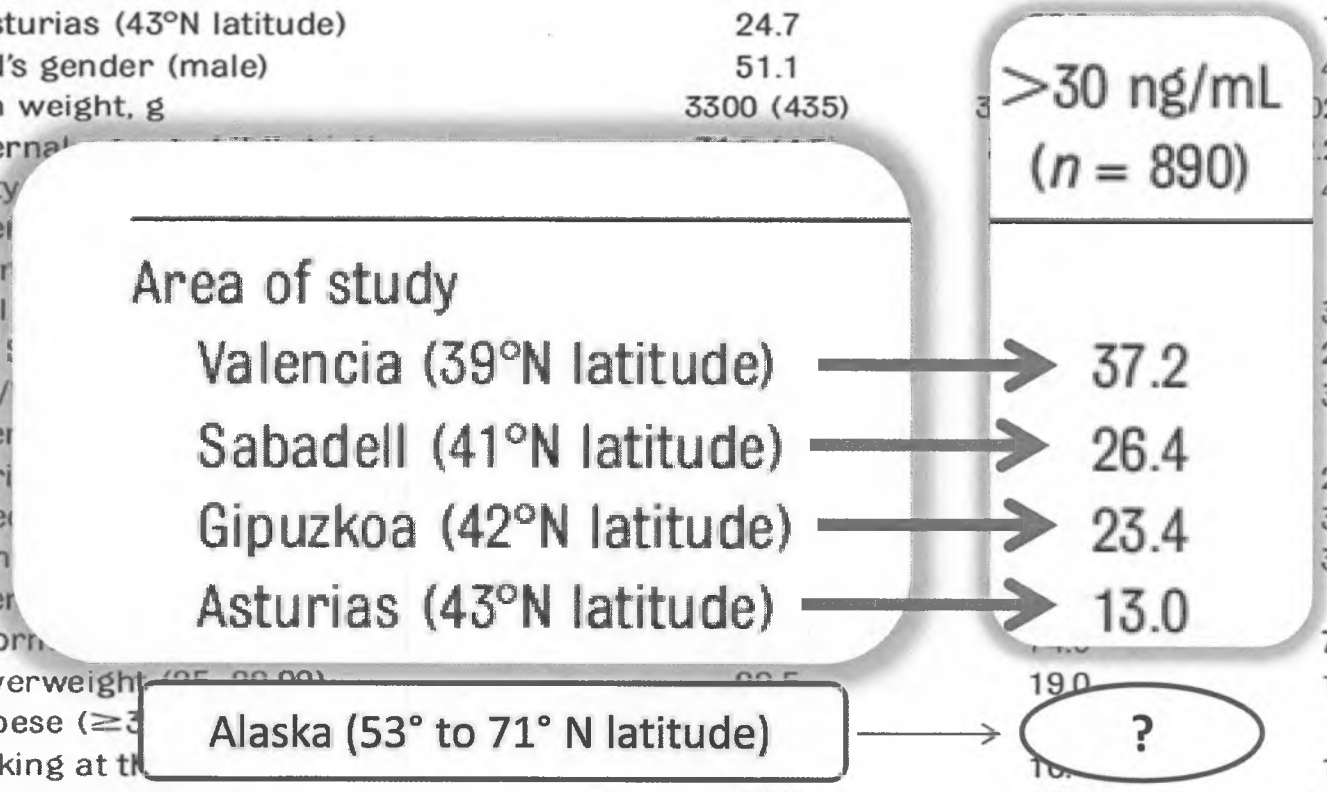
RESULTS: The median plasma value of $25(OH)D_3$ in pregnancy was 29.6 ng/mL (interquartile range, 21.8–37.3). A positive linear relationship was found between circulating concentrations of maternal $25(OH)D_3$ concentrations in pregnancy and mental and psychomotor scores in the offspring. After adjustment for potential confounders, infants of mothers with $25(OH)D_3$ concentrations in pregnancy >30 ng/mL showed higher mental score (β = 2.60; 95% CI 0.63–4.56) and higher psychomotor score (β = 2.32; 95% CI 0.36–4.28) in comparison with those of mothers with $25(OH)D_3$ concentrations <20 ng/mL.

CONCLUSIONS: Higher circulating concentration of maternal $25(OH)D_3$ in pregnancy was associated with improved mental and psychomotor development in infants. *Pediatrics* 2012;130:e913–e920

TABLE 1 Characteristics of Participants According to Maternal Circulating 25(OH)D_3 Concentrations in Pregnancy

	Serum 25(OH)D_3 Concentration			*P* Value Trend
	<20 ng/mL (*n* = 356)	20–30 ng/mL (*n* = 574)	>30 ng/mL (*n* = 890)	
Area of study				
Valencia (39°N latitude)	20.2	29.6	37.2	<.001
Sabadell (41°N latitude)	30.3	23.2	26.4	
Gipuzkoa (42°N latitude)	24.7	27.2	23.4	
Asturias (43°N latitude)	24.7		13.0	
Child's gender (male)	51.1		48.8	.722
Birth weight, g	3300 (435)		2 (419)	.685
Maternal			.2 (4.0)	.004
Parity			44.3	.053
Mate			7.4	.460
Parer				
I/II			36.0	.037
III			26.5	
IV/			37.5	
Mater				
Pri			22.3	.273
Se			39.4	
Un			38.3	
Mater				
Norm			75.6	.086
Overweight		19.0	16.6	
Obese (≥3			7.8	
Smoking at t			13.9	.022
Alcohol during pregnancy (yes)	18.0	16.4	22.4	.013

Values are percentages for categorical variables and mean (SD) for continuous variables.



A positive linear relationship was found between circulating concentrations of maternal 25(OH)D_3 in pregnancy and both mental (Fig 3A) and psychomotor (Fig 3B) development scores in the offspring. In multivariable models, each 10 ng/mL increase in 25(OH)D_3 in pregnancy resulted in up to 0.79 and 0.88 points increase in mental and psychomotor development scores in offspring, respectively (Table 2). In the basic model with adjustment for area of study, infants of mothers with 25(OH)D_3 concentrations >30 ng/mL showed an advantage of 3.17 and 2.42 points in the mental and psychomotor scores, respectively, in comparison with those of mothers with 25(OH)D_3 concentrations <20 ng/mL (model 1) (Table 2). Although attenuated, these associations remained significant after adjustment for potential confounders including child's gender, birth weight, maternal country of origin, maternal age, parental socioeconomic status, maternal education level, parity, maternal pre-pregnancy BMI, and

DISCUSSION

To our knowledge this is one of the first large-scale prospective pregnancy cohort studies to examine the association between maternal circulating 25(OH)D_3 concentrations in pregnancy and offspring neuropsychological development in infancy. Higher concentrations of circulating 25(OH)D_3 in pregnancy were associated with improved mental and psychomotor scores. Infants of mothers with 25(OH)D_3 concentrations >30 ng/mL (clinically considered as optimal levels) showed an advantage of 2.6 and 2.3 points in mental and psychomotor scores, respectively, in comparison with those of mothers with 25(OH)D_3 concentrations <20 ng/mL (considered as deficient levels). The association remained significant after adjusting for a wide range of potential confounding and intermediate factors.

The main strengths of this study include its population-based prospective design and large sample size as well as examination of the associations with

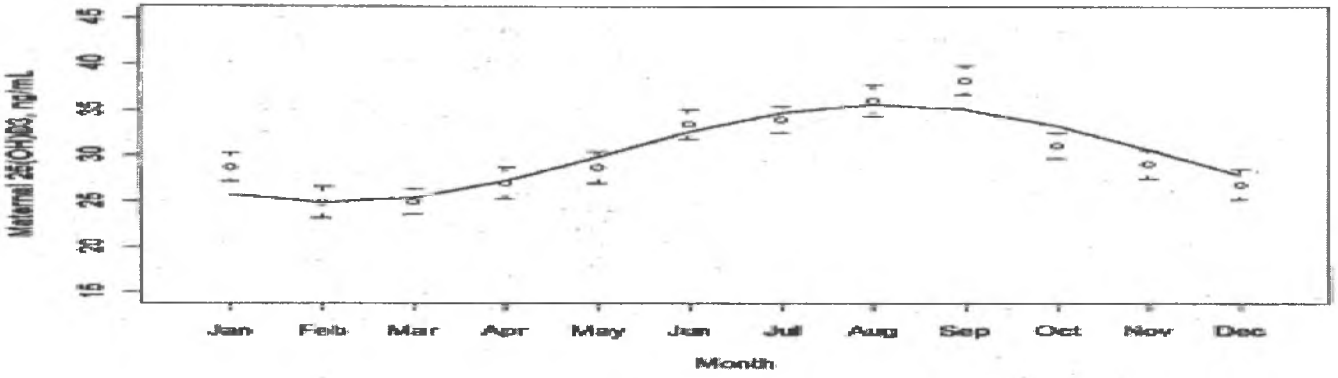


FIGURE 2
Fitted sinusoidal model for observed maternal circulating concentration of 25(OH)D_3 superimposed on plot of observed monthly mean and 95% CI values for 25(OH)D_3 concentration among 2112 participants in the INMA Project.

Emphasis marks and distributed by Representative Seaton

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High Prevalence of Vitamin D Insufficiency in Black and White Pregnant Women Residing in the Northern United States and Their Neonates[1]

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Abstract

In utero or early-life vitamin D deficiency is associated with skeletal problems, type 1 diabetes, and schizophrenia, but the prevalence of vitamin D deficiency in U.S. pregnant women is unexplored. We sought to assess vitamin D status of pregnant women and their neonates residing in Pittsburgh by race and season. Serum 25-hydroxyvitamin D (25(OH)D) was measured at 4–21 wk gestation and predelivery in 200 white and 200 black pregnant women and in cord blood of their neonates. Over 90% of women used prenatal vitamins. Women and neonates were classified as vitamin D deficient [25(OH)D <37.5 nmol/L], insufficient [25(OH)D 37.5–80 nmol/L], or sufficient [25(OH)D > 80 nmol/L]. At delivery, vitamin D deficiency and insufficiency occurred in 29.2% and 54.1% of black women and 45.6% and 46.8% black neonates, respectively. Five percent and 42.1% of white women and 9.7% and 56.4% of white neonates were vitamin D deficient and insufficient, respectively. Results were similar at <22 wk gestation. After adjustment for prepregnancy BMI and periconceptional multivitamin use, black women had a smaller mean increase in maternal 25(OH)D compared with white women from winter to summer (16.0 ± 3.3 nmol/L vs. 23.2 ± 3.7 nmol/L) and from spring to summer (13.2 ± 3.0 nmol/L vs. 27.6 ± 4.7 nmol/L) ($P < 0.01$). These results suggest that black and white pregnant women and neonates residing in the northern US are at high risk of vitamin D insufficiency, even when mothers are compliant with prenatal vitamins. Higher-dose supplementation is needed to improve maternal and neonatal vitamin D nutriture. J. Nutr. 137: 447–452, 2007.

Babies at birth deficient or insufficient = 92.4% = 66.1%

Introduction

Rickets, once thought to have been nearly eradicated in the United States in the 1930s (1), has again become a major public health problem. Several reports have been published describing recent cases of rickets in infants, most of whom were black and exclusively breastfed (2–5). The reemergence of rickets is thought to be due to an epidemic of vitamin D deficiency in mothers and children (6). A newborn's vitamin D stores are completely reliant on vitamin D from the mother (7). Not surprisingly, poor maternal vitamin D status during pregnancy is a major risk factor for infant rickets (8–10).

In addition to causing poor global mineralization of the skeleton, vitamin D deficiency has implications for numerous other nonskeletal health outcomes. In utero or early life vitamin D deficiency has been linked to an increased risk of type 1 diabetes (11), asthma (12), and schizophrenia (13,14). Fascinating new data also show that vitamin D regulates placental development and function (15), which suggests that maternal vitamin D status may be associated with adverse outcomes of pregnancy, such as miscarriage, preeclampsia, and preterm birth.

The most important source of vitamin D is the skin's synthesis of the vitamin from UV B solar radiation (16). Any process that reduces UV B photons from entering the epidermis will diminish cholecalciferol (vitamin D-3) production. The skin pigment melanin absorbs UV B photons and can reduce vitamin D-3 synthesis by >90% (17). Consequently, African Americans are at high risk of vitamin D deficiency. The most recent data from the National Health and Nutrition Examination Survey (1988–1994) indicated that vitamin D deficiency [25-hydroxyvitamin D [25(OH)D] ≤37.5 nmol/L] was prevalent in 42% of black childbearing-aged women and only 4% of white childbearing-aged women residing throughout the United States (18). Vitamin D status is also worsened in winter months (November through March), when, at latitudes above 37°, less UV B radiation reaches the earth and little or no vitamin D can be synthesized in the skin (16,19). Indeed, vitamin D deficiency in U.S. childbearing-aged women was more than 3 times as common in winter than summer in both blacks and whites (18).

Despite the striking racial disparity in vitamin D deficiency and the strong influence of season, there are few recent investigations into the vitamin D status of U.S. black and white pregnant women and their neonates throughout the year. Given

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0022-3166/07 $8.00

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TABLE 2 Vitamin D status of white and black pregnant women and their neonates[1]

	White women, $n = 200$	Black women, $n = 200$
4–21 wk gestation		
Serum 25(OH)D,[2] *nmol/L*	73.1 (69.4, 76.9)	40.2 (37.9, 42.7)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	2.0	44.9**
Insufficient: 25(OH)D 37.5–80 nmol/L	60.3	51.0
Sufficient: 25(OH)D >80 nmol/L	37.3	4.1
37–42 wk gestation		
Serum 25(OH)D, *nmol/L*	80.4 (76.0, 85.1)	49.4 (46.1, 52.9)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	5.0	29.2**
Insufficient: 25(OH)D 37.5–80 nmol/L	41.2	54.1
Sufficient: 25(OH)D >80 nmol/L	53.8	16.7
Cord blood		
Serum 25(OH)D, *nmol/L*	67.4 (63.8, 71.3)	39.0 (36.3, 41.8)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	9.7	45.6**
Insufficient: 25(OH)D 37.5–80 nmol/L	56.4	46.8
Sufficient: 25(OH)D >80 nmol/L	33.9	7.6

[1] Values are geometric means [95% CI] or %. *Different from white women, $P < 0.001$ (student's t test); **different from white women, $P < 0.001$ (chi-square test).
[2] Log-transformed to ensure normality.

Arch Dis Child. 2013 Jan 8. [Epub ahead of print]

Vitamin D status of exclusively breastfed infants aged 2-3 months.

Wall CR, Grant CC, Jones I.

Source: Discipline of Nutrition, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand.

Abstract

BACKGROUND: New Zealand in 2008 adopted WHO policy which recommends that all infants are exclusively breast fed until 6 months of age. The benefits of this policy for the infant are undisputed; however, this policy has the potential to adversely impact on infant vitamin D status. A number of countries now recommend that all breastfed infants receive daily vitamin D supplementation of 400 IU to prevent rickets. New Zealand has no policy on the vitamin D supplementation of 'low-risk' breastfed infants. There are no data on the vitamin D status of exclusively breastfed infants in the first few months of life in New Zealand.

AIM: To describe serum 25-hydroxy-vitamin D (25(OH)D) concentrations in exclusively breastfed infants aged 2-3 months.

DESIGN/METHODS: Healthy term exclusively breastfed infants who were receiving no vitamin D supplements were enrolled over a 15-month period. A capillary blood sample was obtained from each infant. Serum 25(OH)D was measured using isotope-dilution liquid chromatography-tandem mass spectrometry.

RESULTS: 94 infants were enrolled (mean age 10 weeks). Median 25(OH)D concentration was 53 nmol/l (IQR 14-100 nmol/l). 23 (24%) infants had serum 25(OH)D concentration <27.5 nmol/l. Infants enrolled during winter had a median (IQR) 25(OH)D serum concentration of 21 nmol/l (14,31). Infants enrolled during summer had a median (IQR) 25(OH)D concentration of 75 nmol/l (55 100) (winter vs summer, p<0.0001).

CONCLUSIONS: Vitamin D deficiency is prevalent in exclusively breastfed infants in New Zealand. Vitamin D supplementation should be considered as part of New Zealand's child health policy.

PMID:23303428[PubMed - as supplied by publisher]

Vitamin D levels of infants converted from nmol/l to the US common measurement for blood serum concentrations, ng/ml:

- -Entire study:
  - Median = 21.2 ng/ml
  - 24% of infants < 11 ng/ml
- -Infants enrolled in winter:
  - Median = 8.4 ng/ml
  - Highest level = 12.4 ng/ml
- -Infants enrolled in summer:
  - Median = 30 ng/ml

Conversion notes by the office of Representative Seaton

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VITAMIN D AND SUICIDE RISK FACTORS

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Abstract

Low vitamin D levels are negatively associated with certain prosuicidal factors such as exacerbation of depression, anxiety, psychosis, and certain medical conditions. Therefore, we hypothesize that they may also be associated with completed suicides. In particular, lower vitamin D levels at the end of winter, secondary to the lower vitamin D production in the skin, (as a result to reduced skin surface exposure as well as reduced duration of exposure, an after effect of uncomfortably low heat index and lower solar radiation). In preparation to test this hypothesis in future research, we now briefly review the existent literature on vitamin D, its deficiency and its reported association with certain risk factors for suicide.

Introduction

Suicide is the 10th leading cause of death worldwide and the second leading cause of death in adolescents and adults ages 15-35 years (1-3). Suicide attempts are 2 to 3 times more likely than fatal completions (4). Approximately 90% of individuals who die by suicide are diagnosable with a psychiatric illness. About 9.5% of the United States population suffers from a mood disorder including 6.7% suffering from major depressive disorder, 18.1% diagnosed with an anxiety disorder and 1.1% with a psychotic disorder expressed by

Vitamin D news

Research reveals link between low vitamin D and military suicide

07 January 2013

Research published this past week is the first to report that low vitamin D levels are associated with an increased risk for suicide in US military personnel.

John C. Umhau, MD, and colleagues in Bethesda, Maryland conducted a prospective, case-control study using serum samples stored in the Department of Defense Serum Repository. The researchers matched 495 verified suicide cases to 495 controls by rank, age and sex.

The researchers found that more than 30% of all participants had vitamin D levels below 20 ng/ml. The subjects with the lowest vitamin D status (<15.5 ng/ml) had the highest risk of suicide, while participants with higher 25(OH)D status showed a decreased risk. The authors conclude,

"Future studies could determine if additional sunlight exposure and vitamin D supplementation might reduce suicide by increasing 25(OH) D levels."

Source:

Umhau JC, et al. Low vitamin D status and suicide: A case-control study of active duty military service members. PLOS ONE. Jan 2013.

Is low vitamin D linked to military suicide?

Posted on January 10, 2013 by John Cannell, MD

The authors studied 495 cases of suicide among active duty military personnel who had their blood drawn within 2 years of their suicide. They compared them to 495 matched cases controls.

Umhau JC et al. Low Vitamin D Status and Suicide: A Case-Control Study of Active Duty Military Service Members. Plos One

More than 30% of the soldiers had vitamin D levels lower than 20 ng/ml, even in the summer. When sampled in the winter, more than 60% of the soldiers had levels less than 20 ng/ml. They then grouped the soldiers in octiles; in other words, they divided the soldiers into 8 equal groups by grouping them according to vitamin D levels. **They found that soldiers with the lowest levels of vitamin D were twice as likely to complete suicide as were soldiers with higher levels.**

The authors made the following points in their paper:

Sunlight may exert benefits over and above that of making vitamin D. For instance, sunlight is involved in melatonin physiology and melatonin can affect mood.

Low vitamin D status has recently been connected with, low cognitive performance, psychotic-like symptoms, and depression.

A depressive episode does not always precede suicide. The development of suicidal thoughts can be sudden and occur within 10 minutes of a suicide attempt. Impulsivity plays a major role in military suicides.

Low serotonin occurs during the winter; and as most know, serotonin is popularly thought to be central to feelings of happiness. This fact may confound the relationship between vitamin D levels and risk of suicide.

A recent study found the vitamin D levels of soldiers in basic training in South Carolina fell at the end of 8 weeks of basic training due to the heavy clothing worn by soldiers.

Dr. Umhau and colleagues concluded,

"Studies are urgently needed to develop an appropriate strategy to insure that service members do not suffer the ill effects of a preventable deficiency of vitamin D."

We agree but would add that the military should take immediate steps to treat vitamin D deficiency that is rampant among their soldiers.

BMJ open

Vitamin D_3 supplementation in patients with frequent respiratory tract infections: a randomised and double-blind intervention study

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PB and ACN contributed equally to this study.

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ABSTRACT

Background: Low serum levels of 25-hydroxyvitamin D_3 are associated with an increased risk of respiratory tract infections (RTIs). Clinical trials with vitamin D_3 against various infections have been carried out but data are so far not conclusive. Thus, there is a need for additional randomised controlled trials of effects of vitamin D_3 on infections.

Objective: To investigate if supplementation with vitamin D_3 could reduce infectious symptoms and antibiotic consumption among patients with antibody deficiency or frequent RTIs.

Design: A double-blind randomised controlled trial.

Setting: Karolinska University Hospital, Huddinge.

Participants: 140 patients with antibody deficiency (selective IgA subclass deficiency, IgG subclass deficiency, common variable immune disorder) and patients with increased susceptibility to RTIs (>4 bacterial RTIs/year) but without immunological diagnosis.

Intervention: Vitamin D_3 (4000 IU) or placebo was given daily for 1 year.

Primary and secondary outcome measures: The primary endpoint was an infectious score based on five parameters: symptoms from respiratory tract, ears and sinuses, malaise and antibiotic consumption. Secondary endpoints were serum levels of 25-hydroxyvitamin D_3, microbiological findings and levels of antimicrobial peptides (LL-37, HNP1–3) in nasal fluid.

Results: The overall infectious score was significantly reduced for patients allocated to the vitamin D group (202 points) compared with the placebo group (249 points; adjusted relative score 0.771, 95% CI 0.604 to 0.985, p=0.04).

Limitations: A single study centre, small sample size and a selected group of patients. The sample size calculation was performed using p=0.02 as the significance level whereas the primary and secondary endpoints were analysed using the conventional

ARTICLE SUMMARY

Article focus

- Recent evidence suggests that vitamin D_3 has potent extraskeletal effects, such as suppression of inflammation and strengthening of mucosal immunity by induction of antimicrobial peptides.
- Data from observational studies suggest that low levels of 25-hydroxyvitamin D_3 are associated with an increased risk of respiratory tract infections.
- Results from a limited number of randomised controlled trials on the protective role of vitamin D_3 against respiratory tract infections are inconclusive and thus additional studies are warranted.

Intervention: Vitamin D_3 (4000 IU) or placebo was given daily for 1 year.

- The main conclusion is that vitamin D_3 supplementation reduces symptoms and antibiotic consumption among patients with an increased frequency of respiratory tract infections. Thus, vitamin D_3 supplementation may be an alternative strategy to reduce antibiotic use among patients with recurrent respiratory tract infections.

Strengths and limitations of this study

- A high daily dose of vitamin D_3 was used, the study time was a full year covering all seasons and patients with an increased frequency of respiratory tract infections were studied.
- A single study centre, small sample size (n=140) and a selected group of patients.

INTRODUCTION

Vitamin D was discovered when it was noted that rachitic children were improved by expos-

but a reduction of *S aureus* and fungal species that often colonise the airways was observed. This could be explained by specific effects by vitamin D_3 on immunity against *S aureus*. In fact, vitamin D_3 induces human β-defensin-2 (HBD-2) with bactericidal activity against *S aureus*.[29] A recent study showed that low vitamin D_3 levels were associated with an increased risk of being colonised by this bacterium.[30] Further, vitamin D_3 affects immunity against *C albicans*, which indicates direct effects of vitamin D_3 on human immunity.[31] Alternatively, it is possible that vitamin D_3 may have prevented symptomatic viral infections, which prompted patients to leave a bacterial sample from the airways. Interestingly, there is both mechanistic and clinical evidence that vitamin D_3 can prevent viral infections,[32–34] although we did not address this in the current study.

Notably, we observed a prominent increase in the serum concentration of 25-hydroxyvitamin D_3, which indicated good compliance and tolerability of the study drug. In fact, there was a trend towards adverse events being reported more often in the placebo group, suggesting that vitamin D_3 possibly could be efficient against other diseases, but this observation requires further studies. No clinically relevant changes of blood chemistry (calcium, phosphate, albumin or creatine) were observed. Despite few adverse events and high tolerability, 16 exclusions occurred during the study year. The main reason was problems to adhere to the protocol and 6/16 patients dropped out of the study a few weeks. The rest failed to send in [illegible], did not leave blood for monitoring of safety parameters or did not take the study drug. One patient was excluded based on symptoms that could be attributed to vitamin D_3 (facial paraesthesia). However, this patient was later confirmed to have been allocated to placebo.

In summary, we found that supplementation with vitamin D_3 reduced the total infectious score with 47 points per patient (23% reduction in the adjusted analysis) during the study year. The observed reduction was lower than the assumed reduction of 70 points per patient (predefined assumption: 210 points=>140 points; a reduction of 30%) that formed the basis for the power calculation. However, despite the predefined level of a reduction of infectious score by 30% as a clinically meaningful effect, we believe that effects lower than this also could be relevant for the individual patient. We base this line of reasoning on the fact that a reduction of 47 points per patient can be translated into 47 days with cough (47 points), 23 days with ear and sinus symptoms (23×2=46 points) or 9 days with cough and ear symptoms together with malaise and antibiotics (9×5=45 points). In addition, our data indicate that vitamin D_3 supplementation reduces the odds of taking antibiotics by approximately 60% in patients with frequent respiratory tract infections. Thus, supplementation with vitamin D_3 could provide a novel strategy to reduce antibiotic use among high consumers and indirectly prevent the emerging epidemic of bacterial resistance.

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Contributors PB designed the study, collected, analysed and interpreted data, wrote the paper. A-CN designed the study, collected and interpreted data, wrote the paper. SH designed and coordinated the study, collected and interpreted data. RSR carried out experimental work and analysed data. BA analysed and interpreted data, wrote the paper. LB-B analysed and interpreted data, wrote the paper. LE analysed and interpreted data. JL analysed and interpreted data, wrote the paper. JA designed the study, interpreted data, wrote the paper.

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In summary, we found that supplementation with vitamin D_3 reduced the total infectious score with 47 points per patient (23% reduction in the adjusted analysis) during the study year. The observed reduction was

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points). In addition, our data indicate that vitamin D_3 supplementation reduces the odds of taking antibiotics by approximately 60% in patients with frequent respiratory tract infections. Thus, supplementation with vitamin

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Alaska Newborn Vitamin D Assessment

- 10,000 babies
- 1 year
- $300,000

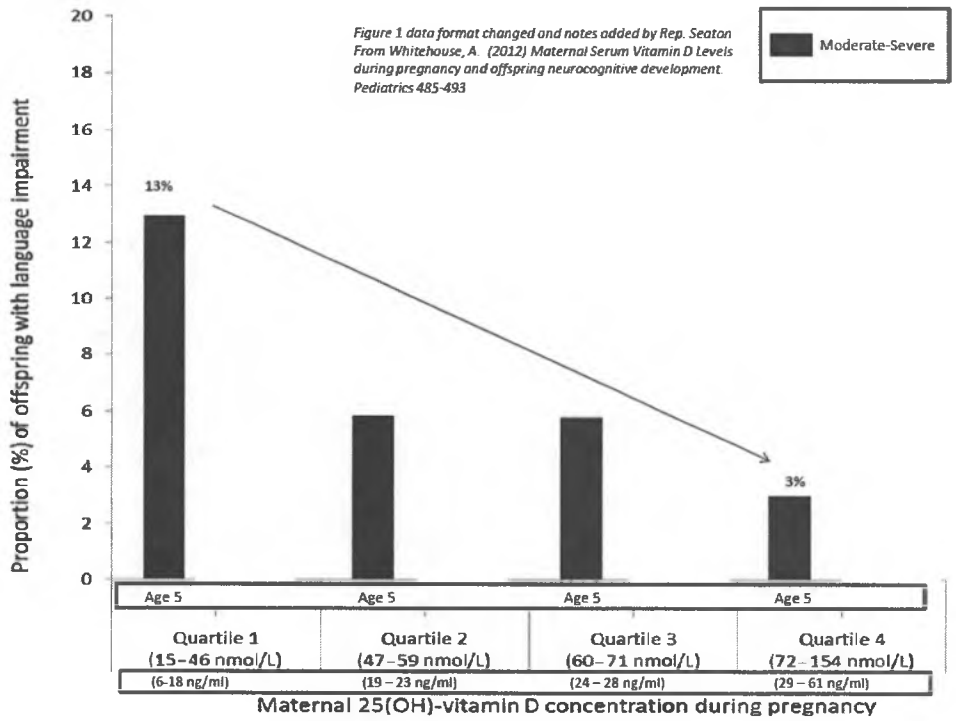
Your Support is Appreciated

POTENTIAL IMPLICATIONS FOR ALASKA

If we assume that Alaska has the same distribution of vitamin D levels as Perth, Australia....

And if every child was then raised to the level of Quartile 4 (29-61 ng/ml) where the rate of moderate-severe language impairment is approximately 3%...

Then, from an Alaskan birth population of 10,000 a year, raising the vitamin D level to above 30 ng/ml could mean 400 fewer children *per year* entering kindergarten with moderate to severe language impairment.



Vitamin D pilot project

The purpose of this project is to attain a baseline of the vitamin D levels in newborns in Alaska. Numerous healthcare organizations and providers recognize the importance to individual health of attaining adequate vitamin D levels. Studies on the vitamin D levels in different populations in Alaska have generally found Alaskans to be deficient. There is a broad body of research that suggests vitamin D deficiency and insufficiency has negative health impacts. Knowledge of the vitamin D levels in Alaska newborns will allow healthcare providers and policy makers to more accurately address, and help prevent the health challenges facing Alaska.

The Alaska State Legislature unanimously passed a resolution establishing prevention of disease as a primary model of health care in Alaska, with a particular emphasis on vitamin D. As there are numerous studies showing the positive benefits of vitamin D, the resolution further encourages the Alaska Department of Health and Social Services and healthcare providers to increase attention to vitamin D deficiency and vitamin D blood testing, and to promote the awareness of the potential benefits of supplementation.

This one-year pilot project would be voluntary on the part of the parents, and would simply add an optional blood test to the two metabolic screens that the State currently requires of newborns. The current mandatory metabolic testing is carried out by heel prick and blood drop method, with the blood collected on a sheet of test paper. As the same technology exists to measure Vitamin D levels, this testing could be carried out at the same time, by the same heel prick. The vitamin D test cards would be sent to lab through an organization that gathers vitamin D level data for different populations.

The cost of the lab test and the individual analysis is approximately $30. 11,320 children were born in Alaska in 2011. However, considering that a number of parents will opt out of the program, the estimate on the test population is about 10,000 newborns. The pilot project could be carried out for $300,000.

Prepared by the Office of Representative Paul Seaton

Vitamin D and Pediatrics

Recent pediatric studies relating to vitamin D levels and infant development have added to growing evidence of the importance of vitamin D sufficiency in neonatal development. The data from these studies strengthens the case for an Alaskan-wide newborn vitamin D testing project.

A 2012 study conducted in Perth, Australia (Whitehouse, et al.) found that maternal blood concentrations of vitamin D during the second trimester of pregnancy had a significant positive association with language outcomes of the offspring, measured at 5 and 10 years of age. The study of 743 Caucasian mother-infant pairs found that for women with a vitamin D insufficiency (equal to or less than 18.4 ng/ml) during pregnancy the risk of having a child with moderate to severe clinical language difficulty was more than twice as great as women with vitamin D serum levels greater than 28 ng/ml. This large scale study shows an association between low maternal serum levels and offspring language impairment.

Another 2012 study conducted among 1820 mother-infant pairs in four locations in Spain (Morales, et al.) found a positive correlation between maternal blood serum concentrations of vitamin D during pregnancy and infant psychomotor and mental scores at 14 months. Infants of mothers with a vitamin D concentration greater than 30 ng/ml showed higher mental and psychomotor scores in comparison with infants of mothers under 20 ng/ml. The statistically significant sample size of this study adds to evidence that prenatal vitamin D levels can affect brain development. This study also offers relevant latitude-based results; the data from the four locations of study, Valencia (at 39°N latitude), Sabadell (at 41°N latitude), Gipuzkoa (at 42°N latitude) and Asturias (43°N latitude), showed that the locations with the northernmost latitudes had the lowest percentages of vitamin D sufficient mothers.

A 2011 study conducted in Philadelphia (Bodnar, et al.) among 200 white mother-infant pairs and 200 black mother-infant pairs found statistically significant difference in rates of vitamin D sufficiency between white and black pairings. At delivery 29.2% of black women were deficient (<15 ng/ml) and 54.1% insufficient (15-32 ng/ml), with black infants 45.6% deficient and 46.8% insufficient. That is compared with 5% and 42.1% of white women and 9.7% and 56.4% white infants.

This could be because African Americans have higher levels of the skin pigment melanin, which absorbs UV B photons and stops the body's creation of vitamin D. Additionally, the study shows that black women had a smaller mean increase in vitamin D levels between spring and summer, supporting the evidence that they are less able to synthesize vitamin D when exposed to typical sunlight. Furthermore, the data shows that over 66% of white infants and 92% of black infants in Philadelphia were vitamin D deficient or insufficient, despite approximately 90% of both groups of pregnant women reporting taking prenatal vitamins.

THE CASE FOR ALASKA: The studies in Australia and Spain (among others) have shown a connection between prenatal vitamin D levels and neural development in children and infants: specifically in language, psychomotor, and mental development. The study in Spain, conducted in four locations, provides evidence that populations at higher latitudes have lower rates of vitamin D sufficiency. The Philadelphia study shows that race (or skin pigmentation) has an effect on the body's levels of vitamin D. Alaska is further north than any of the locations studied in Spain, suggesting our state may have even lower levels of vitamin D sufficiency. Alaska includes diversely pigmented ethnic groups that may have differing levels of vitamin D. The long sleeve clothing worn in Alaska to avoid mosquitoes may also imitate the solar shield of pigmentation, leading to vitamin D insufficiency.

If we establish a project in Alaska to test newborn vitamin D levels across a wide spectrum of locations and population groups, the collected data would provide insight into how our populations are affected by Alaska's northern latitude and which subgroups are at a greater risk of vitamin D deficiency. If results show that Alaskan newborns are vitamin D deficient, the implications of neurodevelopmental effects from studies such as Australia (Whitehouse et al.) and Spain (Morales et al.) may help explain why some of our education strategies have not yet yielded the results we expect.

Emphasis marks and distributed by Representative Seaton

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Australia

Language Impairment

Maternal Serum Vitamin D Levels During Pregnancy and Offspring Neurocognitive Development

Andrew J. O. Whitehouse, Barbara J. Holt, Michael Serralha, Patrick G. Holt, Merci M. H. Kusel and Prue H. Hart

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Maternal Serum Vitamin D Levels During Pregnancy and Offspring Neurocognitive Development

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KEY WORDS
vitamin D, neurocognitive, language impairment, behavioral problems, emotional problems, Raine study

ABBREVIATIONS
95% CI—95% confidence interval
CBCL—Child Behavior Checklist
OR—odds ratio
PPVT-R—Peabody Picture Vocabulary Test—Revised

Ms Kusel and Dr Hart contributed equally to this work.

Drs Whitehouse, Kusel, and Hart developed the hypotheses; Ms Holt, Mr Serralha, Dr Holt, and Dr Hart analyzed serum samples for 25(OH)-vitamin D concentrations; and Dr Whitehouse conducted the statistical analyses and wrote the main drafts of the manuscript. All authors contributed to the interpretation and discussion of the results and other sections of the manuscript.

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WHAT'S KNOWN ON THIS SUBJECT: Vitamin D levels in the general population have decreased considerably over the past decade. The implications of maternal vitamin D insufficiency during pregnancy for offspring neurocognitive development remain unclear.

WHAT THIS STUDY ADDS: Studying a large sample and using a prospective longitudinal design, this study demonstrates a link between maternal vitamin D insufficiency during pregnancy and offspring language impairment. There was no association with childhood behavioral or emotional problems.

abstract

OBJECTIVE: To determine the association between maternal serum 25(OH)-vitamin D concentrations during a critical window of fetal neurodevelopment and behavioral, emotional, and language outcomes of offspring.

METHODS: Serum 25(OH)-vitamin D concentrations of 743 Caucasian women in Perth, Western Australia (32°S) were measured at 18 weeks pregnancy and grouped into quartiles. Offspring behavior was measured with the Child Behavior Checklist at 2, 5, 8, 10, 14, and 17 years of age (n range = 412–652). Receptive language was assessed with the Peabody Picture Vocabulary Test—Revised at ages 5 (n = 534) and 10 (n = 474) years. Raw scores were converted to standardized scores, incorporating cutoffs for clinically significant levels of difficulty.

RESULTS: χ^2 analyses revealed no significant associations between maternal 25(OH)-vitamin D serum quartiles and offspring behavioral/emotional problems at any age. In contrast, there were significant linear trends between quartiles of maternal vitamin D levels and language impairment at 5 and 10 years of age. Multivariate regression analyses, incorporating a range of confounding variables, found that the risk of women with vitamin D insufficiency (≤46 nmol/L) during pregnancy having a child with clinically significant language difficulties was increased close to twofold compared with women with vitamin D levels >70 nmol/L.

CONCLUSIONS: Maternal vitamin D insufficiency during pregnancy is significantly associated with offspring language impairment. Maternal vitamin D supplementation during pregnancy may reduce the risk of developmental language difficulties among their children. *Pediatrics* 2012:129:485–493

Maternal vitamin D insufficiency during pregnancy is associated with adverse health outcomes in offspring, including intrauterine growth-restriction,[1] reduced bone-mineral accrual[2] and recurrent wheeze.[3] To date, the majority of evidence for an effect of maternal vitamin D levels on fetal brain development has come from studies investigating the timing of pregnancies, which have identified an increased risk for multiple sclerosis among the offspring of pregnancies in which the first and second trimesters coincided with the winter and spring months.[4] Direct sunlight is a main source of vitamin D, and it has been hypothesized that maternal vitamin D insufficiency (most common in winter and spring) during the first and second trimesters, may underpin the association between the timing of pregnancy and offspring morbidity.[4,5] However, it is often difficult in these observational studies to investigate sunlight exposure and consequent vitamin D levels in isolation from other seasonal factors that may also influence fetal neurodevelopment, such as changes in temperature, and maternal nutrition and infection.[6]

Vitamin D from the skin and diet is metabolized in the liver to 25(OH)-vitamin D, which can then be measured in blood samples as a more direct investigation of vitamin D status.[5] Although rodent studies have linked low maternal 25(OH)-vitamin D during pregnancy with atypical behavior among pups,[7,8] only 1 study has examined this association in humans. Gale and colleagues[9] measured the circulating 25 (OH)-vitamin D levels of 466 pregnant women and reported no statistically significant association with offspring behavior and verbal IQ at 9 years of age. However, the study was limited by considerable sample attrition, with only 178 (38.2%) of the original cohort contributing data at the 9-year-old follow-up. Consequently, there were small numbers of children identified with "problem behaviors" at follow-up (n range: 24–33), which limited the capacity of the study to identify statistically significant effects. Furthermore, maternal vitamin D concentrations were obtained during the third trimester of pregnancy (median: 32.6 weeks; range: 28–42 weeks), which may not reflect circulating levels earlier in gestation, when cortical structures critical to behavioral regulation and language acquisition are first formed.[10]

Given that vitamin D insufficiency is observed in up to 60% of Caucasian women[11] and that the rate among women with dark skin is estimated to be even higher,[12–14] understanding the implications for offspring neurodevelopment is of urgent importance. Here we report a large-scale longitudinal study of the association between maternal 25(OH)-vitamin D concentrations measured during the second trimester and behavioral and language development of offspring to age 17 years. Participants were from the Western Australian Pregnancy Cohort (Raine) Study, a sample of pregnant women and their offspring from Perth, Western Australia, which is at latitude 32°S. Given the relatively few number of non-Caucasian participants in the Raine Study and recent findings that ethnicity may confound both 25(OH)-vitamin D serum concentrations and cognitive test scores,[15,16] the current study investigated Caucasian mothers and their offspring only.

METHODS

Participants

The Western Australian Pregnancy Cohort (Raine) Study recruited pregnant women from the public antenatal clinic at King Edward Memorial Hospital or surrounding private clinics in Perth (Australia) between May 1989 and November 1991 (n = 2900). The inclusion criteria were a gestational age between 16 and 20 weeks, English language skills sufficient to understand the study demands, an expectation to deliver at King Edward Memorial Hospital, and an intention to remain in Western Australia to enable future follow-up of their child.[17] Participant recruitment and all follow-ups of the study families were approved by the Human Ethics Committees at King Edward Memorial Hospital and/or Princess Margaret Hospital for Children in Perth, Western Australia. Parents provided written informed consent to participate at each follow-up. The current study included only those mother-child dyads in which the mother self-identified as Caucasian and where maternal blood was collected at 18 weeks' gestation and behavioral data were available for the offspring.

Maternal 25(OH)-Vitamin D

From 1989 to 1991, venous blood was obtained at 18 weeks' pregnancy in 929 randomly selected women, centrifuged, and serum collected and stored at −80°C. In June 2011, Serum 25(OH)-vitamin D levels were measured using an enzyme immunoassay kit from Immunodiagnostic Systems Ltd (Scottsdale, AZ). Vitamin D concentrations in stored sera have been shown to remain stable for >3 decades.[18,19] Twenty-eight samples were also measured by using isotope-dilution liquid chromatography-tandem mass spectrometry by RMIT Drug Discovery Technologies (Melbourne, Australia) according to published methodology.[20] A correlation of 25(OH)-vitamin D concentrations for samples assayed by both techniques was strong (r^2 = .87) and confirmed that there were no molecules (vitamin D metabolites or otherwise) in sera of 18-week pregnant women that interfered with the immunoassay of 25(OH)-vitamin D. The assay of 25(OH)-vitamin D by isotope-dilution liquid chromatography-tandem

mass spectrometry gave concentrations of 25(OH)-vitamin D that were slightly higher than those measured by immunoassay (slope 0.95 ± 0.07). Overestimation of 25(OH)-vitamin D by the former assay has recently been reported.[21] For these reasons, the 25(OH)-vitamin D levels in the serum from pregnant women have been divided into quartiles, which because of the strong correlative value was not influenced by the assay used for the measure of serum 25(OH)-vitamin D concentration.

Behavioral Development

The Child Behavior Checklist (CBCL), an empirically validated measure of child behavior by parent report, was used to measure child and adolescent behavior. The CBCL for Ages 2–3 (CBCL/2–3)[22] was used at the 2-year follow-up, and the CBCL for Ages 4–18 (CBCL/4–18)[23] was administered at the 5-, 8-, 10-, 14-, and 17-year follow-ups. These measures contain a list behavioral/emotional problem items (CBCL/2–3: $n = 99$; CBCL/4–18: $n = 118$) that parents rate as not true (score of 0), somewhat or sometimes true (score of 1), or very or often true (score of 2) of their children. Both measures are widely used in the research literature and show good internal reliability and validity in a number of population settings.[23] A clinical calibration with Australian children demonstrated moderately high sensitivity (83% overall) and specificity (67% overall) to a clinical diagnosis, and good test-retest reliability.[24] The 3-year predictive validity of the CBCL/2–3 for CBCL/4–18 outcomes across both genders is $r = .49$, indicating moderate predictive power.[19]

The raw scores produced by the CBCL/2–3 and CBCL/4–18 were converted into t scores (standardized by age and gender) for total, internalizing, and externalizing behavior. The recommended clinical cutoff score ($t \geq 60$) was applied to the CBCL t scores, to obtain 3 binary variables indicative of clinically significant total, internalizing, and externalizing problems.[23] The term "clinically significant" refers here to maladaptive behavior that falls within a defined clinical range for behavioral problems.[22]

Language Development

The Peabody Picture Vocabulary Test—Revised (PPVT-R)[25] was administered to the children at the 5- and 10-year follow-ups, providing a widely used measure of receptive vocabulary. Because of a lack of Australian norms, raw scores were converted to z scores, which were then used to identify children with mild (z score between −1 and −1.5) or moderate-severe (z score <1.5) language impairment.[26,27] The PPVT-R correlates well with the vocabulary subtests of the Stanford-Binet Intelligence Scales ($r = .72$) and the Wechsler Intelligence Scale for Children ($r = .69$).[25]

Sample Characteristics

A range of variables were investigated to determine whether the participants in the current study were representative of the broader Raine cohort. These included sociodemographic factors recorded at 18 weeks' pregnancy (maternal race/ethnicity; maternal age at conception, maternal education, family income, presence of biological father in the family home); antenatal variables recorded at 34 weeks' pregnancy (maternal smoking and alcohol consumption during pregnancy); and obstetric variables recorded at birth (gestational age, offspring gender, parity, Apgar scores 5 minutes after birth).

Statistical Analyses

Because there is currently no standard definition of optimal levels of vitamin D,[5] we divided maternal 25(OH)-vitamin D concentrations into quartiles, which is a well-established practice within the field.[5,9,16,28] The current study was primarily interested in developmental problems among offspring, and thus all outcome variables were examined categorically, incorporating thresholds for clinical levels of difficulty on the CBCL ($t \geq 60$) and PPVT-R (mild difficulty: z score between −1 and −1.5; moderate-severe difficulty <1.5). χ^2 linear-by-linear trends were examined to determine the effect of in utero exposure to increasing levels of maternal 25(OH)-vitamin D. Significant effects were followed up by using generalized estimating equations, which enabled a longitudinal investigation of the influence of maternal 25(OH)-vitamin D concentrations on offspring performance on that particular scale across multiple ages. For generalized estimating equations modeling, we adopted a 3-stage procedure: model 1 investigated the effect of maternal 25(OH)-vitamin D concentration on the longitudinal outcome variable; model 2 included any confounder in which χ^2 analyses revealed a main effect on the independent variable at the conservative α level of $P < .20$[29]; and model 3 included the same confounders as model 2 in addition to a variable denoting the season in which maternal blood was collected to determine whether any effect was specific to maternal 25(OH)-vitamin D levels rather than other seasonal factors. For each model, odds ratios (OR) and 95% confidence intervals (95% CI) are reported. The α level for all analyses was $P < .05$. The analyses were conducted separately for each gender, and highly similar patterns and magnitudes of findings were observed.

RESULTS

Sample Characteristics

Among the 929 women who had maternal blood obtained at 18 weeks' pregnancy and later analyzed for 25(OH)-vitamin D levels, 815 (87.7%) self-identified as Caucasian. The 114

TABLE 1 Frequency Characteristics of the Sample in the Current Study According to Quartiles of Maternal Serum 25(OH)-Vitamin D Concentration Obtained at 18 Weeks' Pregnancy

		Maternal 25(OH)-Vitamin D Concentration				*P* Value
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
25(OH)-vitamin D concentration (nmol/L)						
n		187	189	182	185	
M (SD)		36.81 (7.14)	53.12 (3.89)	65.17 (3.37)	83.54 (12.08)	<.01
Range		15–46	47–59	60–71	72–154	
Covariates	*N*	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*P* Value
Maternal age at conception, y	723					.19
<20		12 (6.6)	13 (9.5)	21 (11.7)	15 (8.4)	
20–24		49 (26.9)	41 (22.4)	37 (20.6)	27 (15.5)	
25–29		58 (31.9)	58 (31.7)	43 (23.9)	56 (31.5)	
30–34		43 (23.6)	44 (24.0)	60 (33.3)	60(33.7)	
≥35		20 (11.0)	27 (14.8)	19 (10.6)	20 (11.2)	
Mother completed secondary school	741					.90
Yes		67 (36.0)	64 (33.9)	67 (36.8)	69 (37.5)	
No		119 (64.0)	1265 (66.1)	115 (63.2)	115 (62.5	
Family income above poverty line	706					.11
Yes		95 (52.5)	108 (59.7)	99 (58.6)	108 (61.7)	
No		86 (47.5)	73 (40.3)	70 (41.4)	67 (38.3)	
Biological father living in family home	724					.21
Yes		163 (89.6)	163 (88.6)	151 (83.9)	154 (88.5)	
No		19 (10.4)	21 (11.4)	29 (16.1)	24 (13.5)	
Season in which maternal blood was collected at 18 wk pregnancy	743					<.01
Summer		9 (4.8)	31 (16.4)	47 (25.8)	54 (29.2)	
Autumn		22 (11.8)	40 (21.2)	37 (20.3)	58 (31.4)	
Winter		65 (34.8)	55 (29.1)	29 (15.9)	29 (15.7)	
Spring		91 (48.7)	63 (33.3)	69 (37.9)	44 (23.8)	
Smoking during pregnancy	724					<.05
None		115 (63.2)	131 (71.2)	127 (70.6)	137 (77.0)	
1–5 cigarettes daily		23 (12.6)	18 (9.8)	16 (8.9)	15 (8.4)	
6–10 cigarettes daily		17 (9.3)	12 (6.5)	13 (7.2)	13 (7.3)	
11–15 cigarettes daily		14 (7.7)	11 (6.0)	9 (5.0)	7 (3.9)	
16–20 cigarettes daily		10 (5.5)	9 (4.9)	9 (5.0)	3 (1.7)	
21+ cigarettes daily		3 (1.6)	3 (1.6)	6 (3.3)	3 (1.7)	
Alcohol intake during pregnancy	724					.37
None		99 (54.4)	105 (57.1)	85 (47.2)	85 (47.8)	
Once a week or less		42 (23.1)	42 (22.8)	52 (28.9)	45 (25.3)	
2–6 drinks per week		30 (16.5)	39 (16.3)	37 (20.6)	41 (3.0)	
7–10 drinks per week		4 (2.2)	2 (1.1)	3 (1.7)	6 (3.4)	
11+ drinks per week		7 (3.8)	5 (2.7)	3 (1.7)	1 (0.6)	
Offspring gender	743					
Female		112 (59.9)	118 (62.4)	105 (57.7)	106 (57.3)	.44
Male		75 (40.1)	71 (37.6)	77 (42.3)	79 (42.7)	
Gestational age at birth of offspring	739					.07
<32 wk		4 (2.2)	4 (2.2)	3 (1.7)	2 (1.1)	
32–37 wk		37 (20.3)	37 (20.2)	27 (15.0)	26 (14.7)	
38–40 wk		106 (58.2)	116 (63.4)	121 (67.2)	110 (62.1)	
>41 wk		35 (19.2)	26 (14.2)	29 (16.1)	39 (22.0	
Offspring parity	724					<.05
1		101 (55.5)	95 (51.6)	87 (48.3)	68 (38.2)	
≥2		81 (44.5)	89 (48.4)	93 (51.7)	110 (61.8)	
Offspring Apgar scores 5 min after birth	722					.83
Generally normal		175 (96.2)	174 (95.1)	176 (98.3)	170 (95.5)	
Fairly low		7 (3.8)	9 (4.9)	3 (1.7)	8 (4.5)	
Critically low		0 (0)	0 (0)	0 (0)	0 (0)	

P values are for between-quartile comparisons.

offspring who had a non-Caucasian mother and another 2 Caucasian offspring with Down syndrome were excluded from the study. Seventy of the remaining 813 offspring did not contribute any behavioral data, which left 743 offspring who were investigated in the current study.

Participants in the current study were representative of the wider Raine cohort in terms of sociodemographic and obstetric characteristics ($P > .05$), except that female offspring were proportionately overrepresented in the current study (current study: 59.4%; remainder of Raine cohort: 43.9%; $\chi^2 = 49.72$, $P < .01$). See Supplemental Table 4 for additional details.

Table 1 presents the sample characteristics of the mother-offspring pairs within each 25(OH)-vitamin D concentration quartile. The quartile markers were ≤46 nmol/L, 47–59 nmol/L, 60–71 nmol/L, and ≥72 nmol/L for quartiles 1 to 4, respectively. The quartile markers were similar to those reported in other Western countries.[30–32] Importantly, the lower quartile threshold (≤46 nmol/L) corresponded well with the most widely used definition of vitamin D insufficiency as a 25(OH)-vitamin D concentration <50 nmol/L.[5]

There was an effect of season of maternal blood collection on 25(OH)-vitamin D quartiles ($P < .01$), in which women in quartiles 1 and 2 were more likely to have had blood samples obtained during the winter and spring months. Women in quartile 1 were also more likely to have smoked cigarettes during pregnancy and less likely to have given birth previously.

Bivariate Analyses

There were no significant linear-by-linear associations in the proportion of children scoring above the clinical cutoff for CBCL morbidity (see Table 2). In contrast, there was a significant linear-by-linear association between vitamin D quartiles and the proportion of offspring with language difficulties at age 5, $\chi^2 = 5.27$, $df = 1$, $P < .05$, and age 10, $\chi^2 = 5.64$, $df = 1$, $P < .05$. Figure 1 shows that as maternal 25(OH)-vitamin D levels during pregnancy increased the proportion of mothers who had offspring with mild or moderate-severe language difficulties decreased.

TABLE 2 Number (%) of Children Exceeding the CBCL Clinical Thresholds (t score ≥60) at Each Follow-Up According to Quartiles of Maternal Serum 25(OH)-Vitamin D Concentration at 18 Weeks' Pregnancy

	Maternal 25(OH)-Vitamin D Concentration				P
	Quartile 1[a] (Lowest)	Quartile 2[b]	Quartile 3[c]	Quartile 4[d] (Highest)	
CBCL year 2					
Total	16 (10.7)	23 (15.1)	14 (9.9)	12 (8.1)	.26
Internalizing	13 (8.7)	12 (7.9)	14 (9.9)	11 (7.4)	.85
Externalizing	18 (12.8)	28 (18.4)	19 (13.5)	20 (13.4)	.96
CBCL year 5					
Total	33 (19.8)	33 (21.0)	36 (22.2)	37 (22.3)	.54
Internalizing	21 (12.6)	26 (16.6)	28 (17.3)	29 (17.5)	.22
Externalizing	27 (16.2)	32 (20.4)	32 (19.8)	34 (20.5)	.36
CBCL year 8					
Total	36 (23.1)	32 (21.1)	32 (20.3)	30 (19.1)	.85
Internalizing	34 (21.8)	36 (23.7)	37 (23.4)	27 (13.2)	.34
Externalizing	35 (22.4)	27 (17.8)	28 (17.7)	28 (17.8)	.32
CBCL year 10					
Total	25 (16.3)	29 (18.4)	22 (14.9)	21 (13.5)	.35
Internalizing	26 (17.0)	31 (19.6)	31 (20.9)	23 (14.7)	.68
Externalizing	24 (15.7)	21 (13.3)	19 (12.8)	18 (11.5)	.29
CBCL year 14					
Total	22 (16.2)	21 (15.1)	17 (12.7)	17 (12.6)	.33
Internalizing	17 (12.5)	17 (12.5)	18 (13.4)	17 (12.6)	.91
Externalizing	23 (16.9)	22 (15.8)	22 (16.4)	15 (11.1)	.22
CBCL year 17					
Total	9 (8.8)	10 (9.7)	9 (8.4)	5 (5.0)	.29
Internalizing	12 (11.8)	11 (10.7)	8 (7.5)	9 (9.0)	.74
Externalizing	13 (12.7)	14 (13.6)	12 (11.2)	6 (6.0)	.31

P values are for χ^2 linear-by-linear association tests.
[a] N: age 2 = 150, age 5 = 167, age 8 = 156, age 10 = 153, age 14 = 136, age 17 = 102.
[b] N: age 2 = 152, age 5 = 157, age 8 = 152, age 10 = 158, age 14 = 139, age 17 = 103.
[c] N: age 2 = 141, age 5 = 162, age 8 = 158, age 10 = 148, age 14 = 134, age 17 = 107.
[d] N: age 2 = 149, age 5 = 166, age 8 = 157, age 10 = 156, age 14 = 135, age 17 = 100.

Multivariate Analyses

Table 3 presents the findings from the multivariate analyses. Offspring of quartile 1 women were more than twice as likely to be categorized in a more severe language impairment category (ie, "typical" to mild impairment or mild impairment to moderate/severe impairment) relative to offspring of quartile 4 women (model 1), OR = 2.04, 95% CI = 1.14 to 3.68, $P < .05$. Model 2, which adjusted for maternal age at conception, family income, maternal smoking during pregnancy, and offspring parity (Table 1 for P values < .20), identified a slightly weakened but still significant effect of "low" maternal 25(OH)-vitamin D levels (ie, quartile 1) on offspring language impairment, OR = 1.92, 95% CI = 1.01 to 3.62, $P < .05$. This effect remained after additional adjustment for season of maternal blood collection (model 3), OR = 1.97, 95% CI = 1.00 to 3.92, $P < .05$.

A final, post hoc analysis found that, within quartile 1, there were no significant correlations between the continuous measures of maternal 25(OH)-vitamin D concentration and PPVT-R $z$scores at 5 ($n = 130$, $r = -.03$, $P = .78$) or 10 years ($n = 121$, $r = -.01$, $P = .99$) of age.

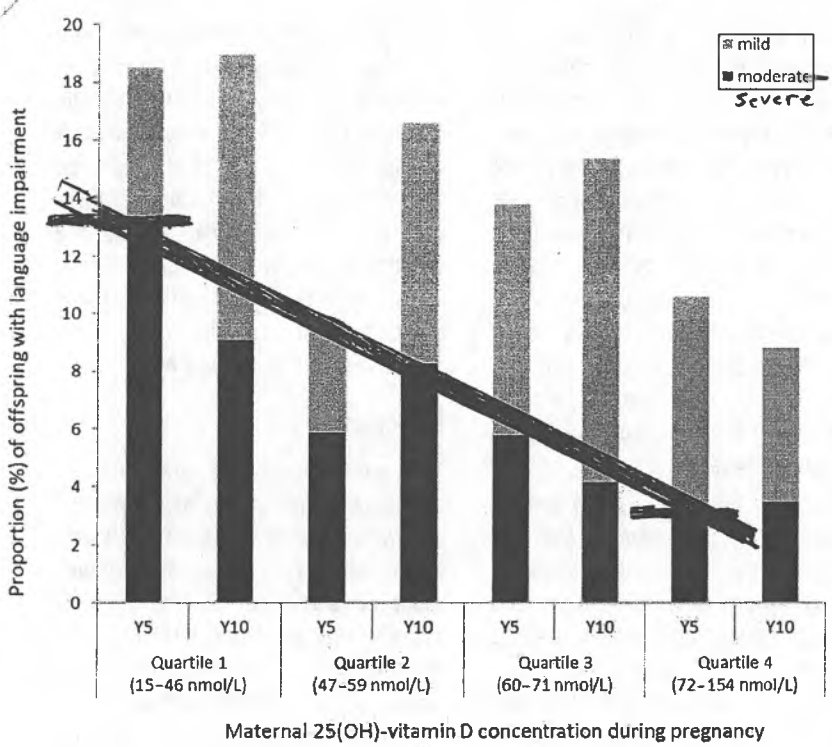

FIGURE 1

The proportion of offspring with mild or moderate-severe language impairment at 5 (Y5)[a] and 10 years (Y10)[b] of age according to maternal serum 25(OH)-vitamin D levels at 18 weeks' pregnancy.

[a] Quartile 1, $n = 130$; quartile 2, $n = 136$; quartile 3, $n = 136$; quartile 4, $n = 132$.

[b] Quartile 1, $n = 121$; quartile 2, $n = 121$; quartile 3, $n = 119$; quartile 4, $n = 113$.

TABLE 3 Generalized Estimating Equations (GEE) Models Showing the Association Between Maternal 25(OH)-Vitamin D Concentration at 18 Weeks' Pregnancy and Offspring Language Impairment During Childhood

Maternal 25(OH)-Vitamin D Concentration	Model 1[a]		Model 2[b]		Model 3[c]	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Quartile 4 (highest)	1.00 (reference)	—	1.00 (reference)	—	1.00 (reference)	—
Quartile 3	1.47 (0.80–2.70)	.21	1.43 (0.75–2.73)	.29	1.44 (0.74–2.80)	.28
Quartile 2	1.28 (0.68–2.40)	.45	1.33 (0.70–2.54)	.39	1.35 (0.71–2.57)	.36
Quartile 1 (lowest)	2.04 (1.14–3.68)	<.05	1.92 (1.01–3.62)	<.05	1.97 (1.00–3.93)	<.05

[a] Unadjusted model.

[b] GEE adjusted for maternal age at conception, family income, maternal smoking during pregnancy, and offspring parity.

[c] GEE adjusted for same confounders as for Model 2, as well as the season of maternal blood collection.

DISCUSSION

This study reports the largest investigation to date of the association between maternal 25(OH)-vitamin D status during pregnancy and offspring neurocognitive development. There was no statistically significant association between maternal 25(OH)-vitamin D concentrations during pregnancy and offspring behavioral and emotional difficulties as measured by the CBCL. This finding replicates the only previous investigation in this area,[9] which revealed no effect of maternal 25(OH)-vitamin D levels on offspring behavior to middle childhood, and extends this null association to adolescence. However, significant linear associations were observed with language impairment at 5 and 10 years of age. The risk of women with serum 25(OH)-vitamin D insufficiency (≤46 nmol/L) during pregnancy having a child with clinically significant language difficulties was increased close to twofold compared with women with 25(OH)-vitamin D levels >70 nmol/L, even after taking into account a range of confounding variables. Importantly, the statistically significant effect was still present after adjusting for the season at which maternal blood was collected, indicating that the association was primarily driven by maternal 25(OH)-vitamin D levels, rather than other seasonal factors.

The developing fetus is completely reliant on maternal vitamin D stores, and thus the concentration of maternal circulating levels at 18 weeks' pregnancy provides an accurate measure of fetal exposure during the second trimester.[31,32] In vitro[33–39] and in vivo[7,40,41] studies have found that vitamin D performs a number of biological functions that are fundamental to neurodevelopment, including a signaling role in neuronal differentiation, a regulation role in the metabolism of neurotrophic factors and neurotoxins, and a protective role during brain inflammation. Vitamin D may also be indirectly involved in fetal brain growth through its role in a number of endocrine functions.[8] Reduced levels of vitamin D may disrupt 1 or more of these functions during critical phases of neurodevelopment. Intriguingly, the current study found evidence for a threshold effect, in that offspring of quartile 1 women were the only group of children at significantly increased risk of language impairment, and within quartile 1, there was no correlation between the continuous 25(OH)-vitamin D and PPVT-R variables. Similar findings have been reported by cross-sectional studies for a range of health outcomes in postnatal life, including bone density, dental health, and colorectal cancers,[42] and together these findings suggest

that a circulating 25(OH)-vitamin D concentration of <50 nmol/L may provide a valid cutpoint to identify vitamin D insufficiency.

The significant association with language ability observed in the current investigation contrasts with the study by Gale and colleagues,[9] which found no effect of maternal vitamin D concentrations on verbal IQ measured by the Wechsler Abbreviated Scale of Intelligence. One possible explanation for this discrepancy is that the current sample (*n* range: 412–652 mother-offspring pairs) was considerably larger than that investigated by Gale et al (178 mother-offspring pairs) and thus provided greater statistical power to identify significant effects. A second possibility relates to the timing of maternal serum sampling, which occurred during the 18th week of pregnancy in the current study but over a later and broader period in the study by Gale et al (median: 32.6 weeks; range: 28–42 weeks). Vitamin D serum concentrations are known to fluctuate according to sunlight exposure and, to a lesser extent, diet.[43,44] Perisylvian structures that subserve much of the neural architecture responsible for language[45] and are anomalous in children with developmental language difficulties,[46,47] including the planum temporal, the pars triangularis and the inferior frontal gyrus, are known to develop during the second and third prenatal trimesters.[48] Although the sampling regimen used in our study was temporally sensitive to second trimester fetal growth, it is possible that 25(OH)-vitamin D serum concentrations measured during the third trimester do not reflect circulating levels during the early stages of perisylvian development. Studies that sample maternal serum at several stages throughout pregnancy will reveal important information about the biological mechanisms underpinning the association between maternal vitamin D levels and fetal neurodevelopment.

Strengths of the study design include the large participant sample, a follow-up period that spanned 2 decades, the assessment of the behavioral and language phenotypes with the same measures at multiple time points, and the direct measurement of maternal 25 (OH)-vitamin D serum levels using enzyme immunoassay and validated by liquid chromatography-tandem mass spectrometry. However, similar to most longitudinal studies, the Raine cohort has experienced a degree of sample attrition over time, such that CBCL data at age 17 was available for just over 50% (*n* = 412) of the original 743 participants eligible for the current study. It is possible that a reduction in the number of participants with age may have influenced the current pattern of findings. Importantly, however, the majority of eligible participants contributed CBCL (82.8%) and PPVT (63.8%) data at age 10, and there is evidence that both behavioral[49,50] and language[51,52] ability in the general population is relatively stable from middle childhood onward. It is also important to highlight that our study included Caucasian participants only, and we caution against extrapolating the findings to non-Caucasian populations. Future studies that investigate large non-Caucasian populations will build on the findings presented here.

CONCLUSIONS

This study found that vitamin D insufficiency among Caucasian women during pregnancy was associated with an increased rate of language impairment among offspring. The findings suggest that the trend over the past decade of a reduction in vitamin D levels among women of reproductive age[11–14] has significant implications for offspring neurodevelopment and public health more generally. Randomized controlled trials of vitamin D supplementation are required to verify these observational data that suggest that an adequate maternal vitamin D status during pregnancy is necessary for optimal language development in offspring.

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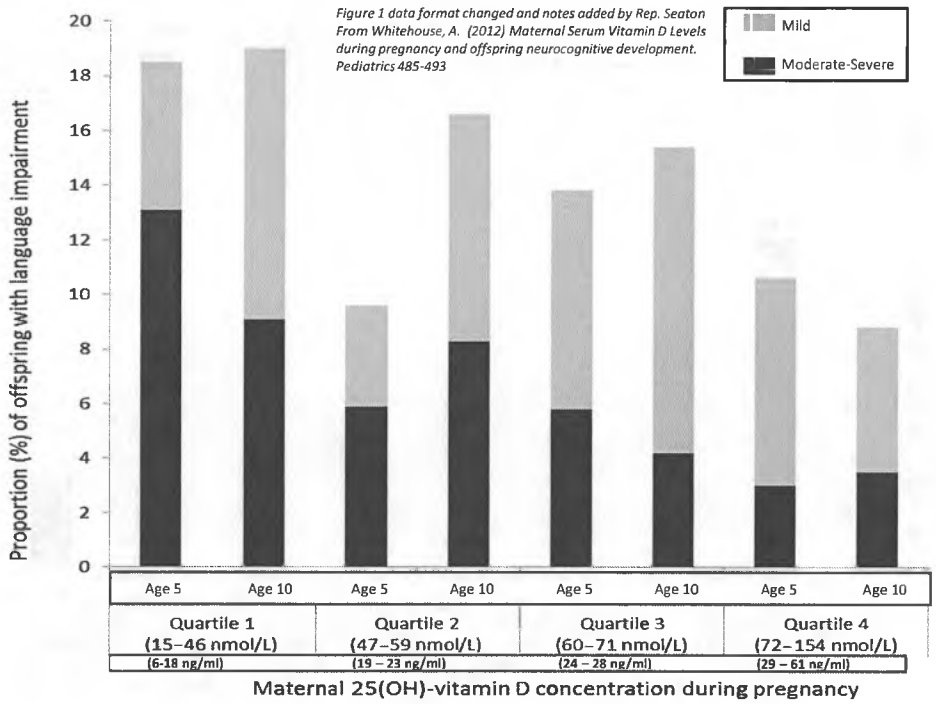
FAIR FARES: *I have a lot of flights scheduled during the next several months. Not only will I be flying to cities along the Atlantic seaboard but to cities in Europe and Asia as well. Trying to determine the best fare is quite challenging. I fly to Philadelphia often enough to know the "sweet spot" for getting the best deal. However, I am not so familiar with the flights to the west coast or Italy. Scrolling through the scores of travel sites only contributes to my confusion. Some quote a fare and will allow me to book a flight directly on their website. Others quote fares but re-direct me to another website to purchase tickets. Some airlines are not listed on the travel sites, so to check on routes and fares I need to go directly to that airline's homepage. More confusing yet is comparing the fares between travel sites, which take different approaches to posting fares. Some post the final cost while others only the baseline fare. The final cost, e.g. including fuel surcharges and landing fees, is not discovered until later stages of the search process. Most sites do not add their own fees to the final cost of the ticket until late in the purchase process. What appears at first glance to be the cheapest ticket, may wind up costing far more than expected. According to an article in* The Wall Street Journal *(The Middle Seat: January 12, 2011), there are a myriad of reasons for the confusion. First, airlines publish fares for every conceivable route and connection. So a travel site looking for a flight between Burlington and Philadelphia can route me through Detroit and Chicago. In a further wrinkle, some sites won't publish fares for routes if it seems unlikely than anyone would actually purchase a ticket for that itinerary. For example, I can usually get a cheaper flight than I would normally take to Philadelphia but I would have to make three stops. Some travel sites publish this itinerary while others do not. So what is the bottom line for travelers? Scan multiple sites. No single site offers the best fares all the time. And as always, if the price is too good to be true, it usually is.*

Noted by WVR, MD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Mild
Moderate-Severe
Proportion (%) of offspring with language impairment
20
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Age 5
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Quartile 1 (15–46 nmol/L) (6-18 ng/ml)
Quartile 2 (47–59 nmol/L) (19 – 23 ng/ml)
Quartile 3 (60–71 nmol/L) (24 – 28 ng/ml)
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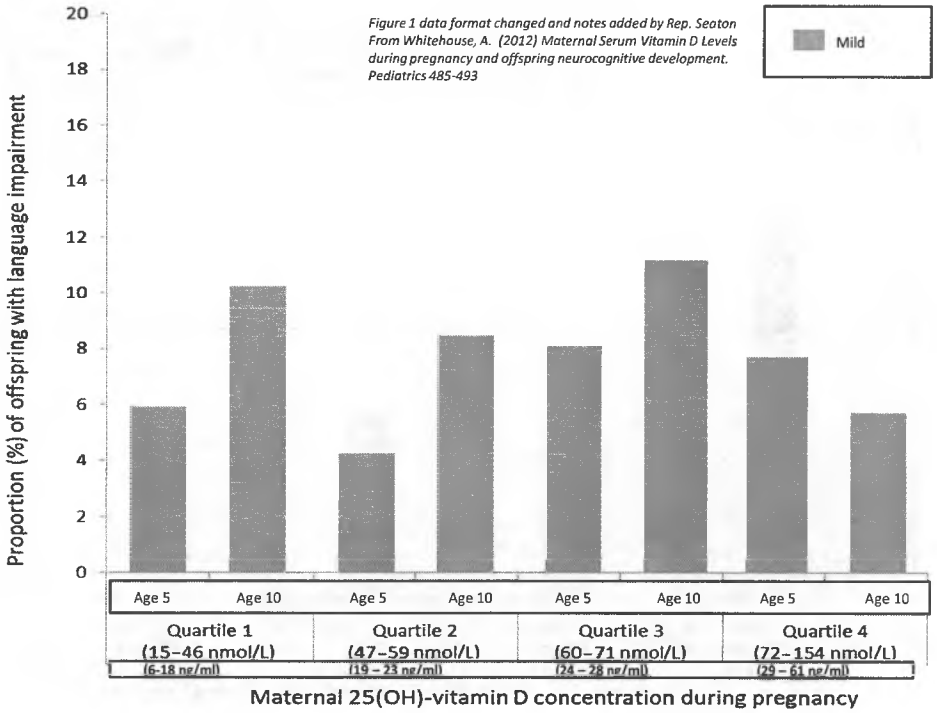
Maternal 25(OH)-vitamin D concentration during pregnancy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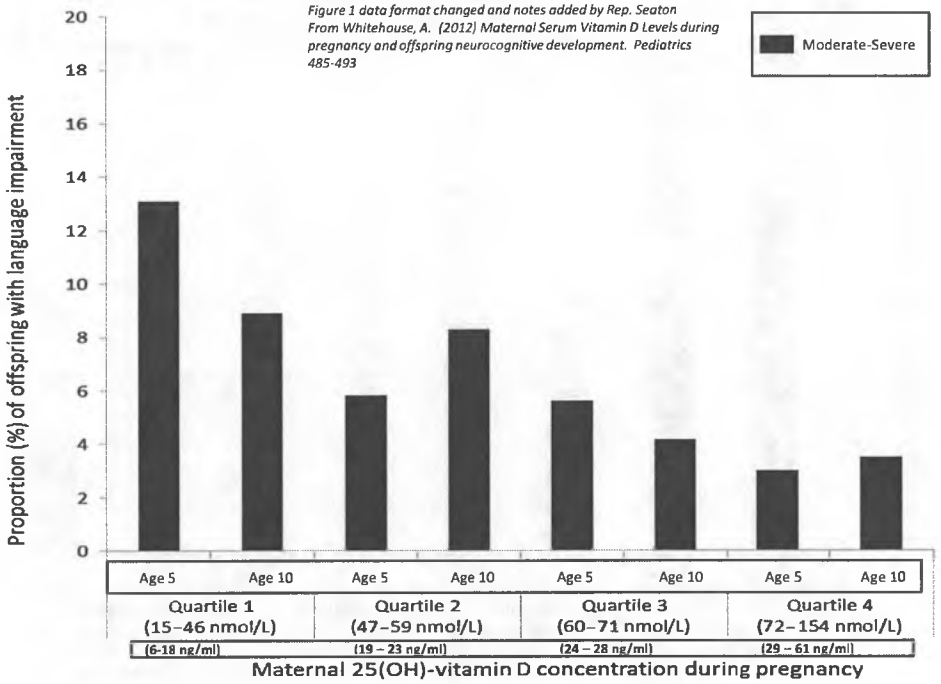
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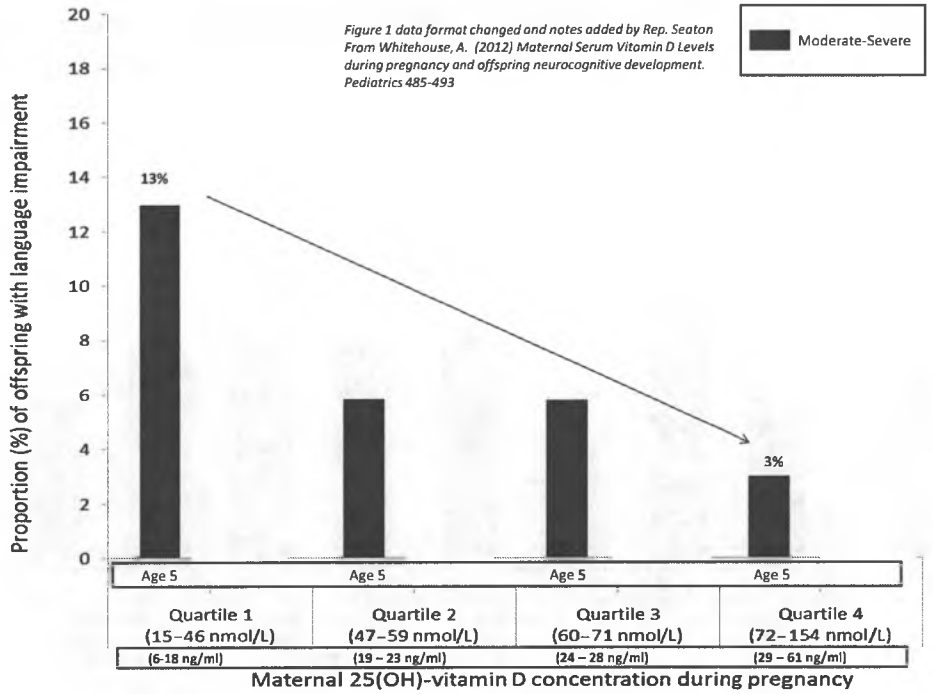
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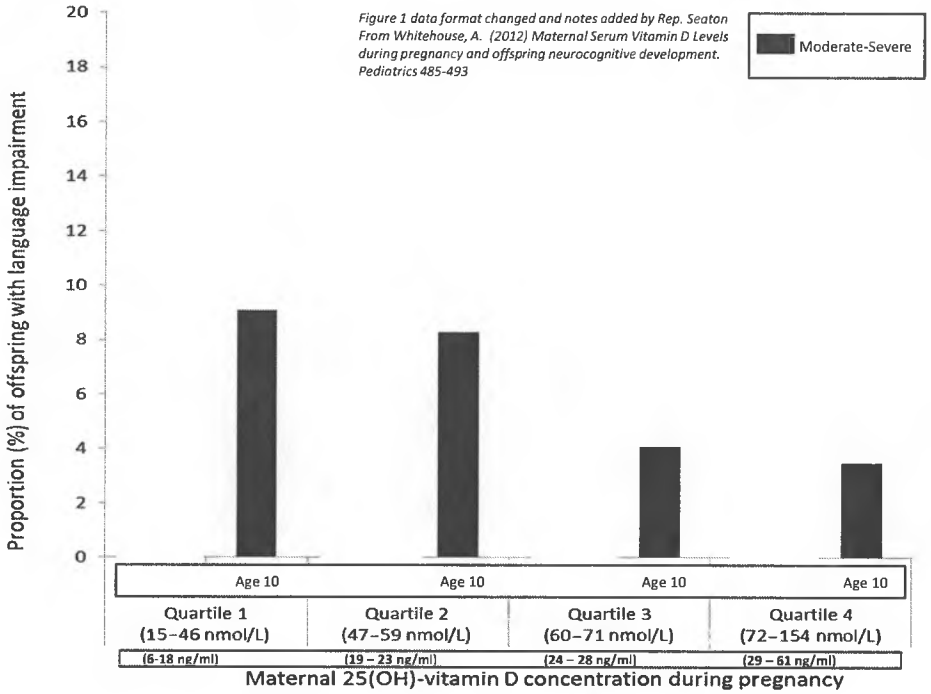
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Circulating 25-Hydroxyvitamin D_3 in Pregnancy and Infant Neuropsychological Development

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KEY WORDS

child development, cognition, infancy, intelligence, vitamin D

ABBREVIATIONS

CI—95% confidence intervals
FP—fractional polynomial
$25(OH)D_3$—25-hydroxyvitamin D_3

*Drs Morales and Guxens contributed equally to this work.

Dr Sunyer had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; Drs Morales, Guxens, Sunyer were responsible for study concept and design; Drs Guxens, Llop, Tardón, Ibarluzea, Espada, and Sunyer were responsible for acquisition of data; Drs Morales and Guxens were responsible for drafting of the manuscript; Drs Llop, Rodríguez-Bernal, Tardón, Riaño, Ibarluzea, Lertxundi, Espada, Rodríguez, and Sunyer were responsible for critical revision of the manuscript for important intellectual content; Dr Guxens was responsible for statistical analysis; and Drs Ibarluzea, Tardón, and Sunyer obtained funding.

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WHAT'S KNOWN ON THIS SUBJECT: Adequate vitamin D status in mothers during pregnancy may influence the health status of offspring later in life. Growing evidence based on animal studies is linking vitamin D to brain development and functioning, but studies in humans are lacking.

WHAT THIS STUDY ADDS: This large-scale prospective pregnancy cohort study examines the association between maternal circulating 25-hydroxyvitamin D_3 concentrations in pregnancy and offspring neuropsychological development. Higher circulating concentration of 25-hydroxyvitamin D_3 in pregnancy was associated with improved mental and psychomotor development in infants.

abstract

OBJECTIVE: To investigate whether circulating 25-hydroxyvitamin D_3 [$25(OH)D_3$] concentration in pregnancy is associated with neuropsychological development in infants.

METHODS: The Spanish population-based cohort study INfancia y Medio Ambiente Project recruited pregnant women during the first trimester of pregnancy between November 2003 and February 2008. Completed data on 1820 mother-infant pairs were used. Maternal plasma $25(OH)D_3$ concentration was measured by high-performance liquid chromatography in pregnancy (mean 13.5±2.1 weeks of gestation). Offspring mental and psychomotor scores were assessed by trained psychologists at age 14 months (range, 11–23) by using the Bayley Scales of Infant Development. β-Coefficients with 95% confidence intervals (CIs) of mental and psychomotor scores associated with continuous or categorical concentrations of maternal plasma $25(OH)D_3$ were calculated by using linear regression analysis.

RESULTS: The median plasma value of $25(OH)D_3$ in pregnancy was 29.6 ng/mL (interquartile range, 21.8–37.3). A positive linear relationship was found between circulating concentrations of maternal $25(OH)D_3$ concentrations in pregnancy and mental and psychomotor scores in the offspring. After adjustment for potential confounders, infants of mothers with $25(OH)D_3$ concentrations in pregnancy >30 ng/mL showed higher mental score (β = 2.60; 95% CI 0.63–4.56) and higher psychomotor score (β = 2.32; 95% CI 0.36–4.28) in comparison with those of mothers with $25(OH)D_3$ concentrations <20 ng/mL.

CONCLUSIONS: Higher circulating concentration of maternal $25(OH)D_3$ in pregnancy was associated with improved mental and psychomotor development in infants. *Pediatrics* 2012;130:e913–e920

Vitamin D deficiency is a public health issue worldwide.[1] Growing evidence based on animal studies is linking vitamin D to brain development and functioning.[2,3] Potential effects of vitamin D during brain development include neurotrophic actions,[4] neuroprotective effects,[5,6] and changes in brain structure and gene expression.[7,8] It is unknown how evidence from animal studies translates to humans, but it is possible that vitamin D status during brain development affects cognitive functioning later in life.

Studies in humans examining the effects of blood concentrations of 25-hydroxyvitamin D_3 [25(OH)D_3] on brain development and cognitive functioning are limited and inconclusive. Several studies in middle-aged and older adults have shown positive associations between serum concentrations of 25(OH)D_3 and cognitive function.[9–18]

Epidemiological studies examining whether circulating concentration of 25(OH)D_3 in early life is associated with brain development and cognitive function are scarce. To date, only 2 prospective studies have assessed the association of vitamin D status in pregnancy, measured as circulating concentrations, with cognitive function in offspring.[19,20] Gale et al[19] found no association between maternal serum 25(OH)D_3 concentrations during late pregnancy (32 weeks) and childhood cognitive function at 9 years of age in 178 mother-child pairs. More recently, a prospective study conducted on 743 mother-child pairs reported insufficient maternal serum 25(OH)D_3 concentrations, measured at 18 weeks pregnancy, to be significantly associated with offspring language impairment at 5 and 10 years of age.[20] Moreover, a larger cross-sectional study (N = 1676) reported null associations of circulating vitamin D concentrations with cognitive function in adolescents (age 12–17 years).[21,22]

Given the emerging evidence on developmental origins of health and disease paradigm,[23] examining the association between vitamin D status in pregnancy and neuropsychological development in early life can be particularly relevant, because windows of unique vulnerability and susceptibility to prenatal influences are inherent in the developing brain more than in the brain of an adult,[24] and consequences of a halted or inhibited developmental process can be permanent owing to little potential for later repair. We assessed whether circulating maternal 25(OH)D_3 concentration in pregnancy, a good marker of vitamin D availability to tissues and reliable indicator of vitamin D status,[25] is associated with offspring neuropsychological development in infancy.

METHODS

Design and Study Population

Data come from 4 prospective population-based pregnant cohort studies in Spain embedded in the INfancia y Medio Ambiente-(Environment and Childhood) Project (www.proyectoinma.org).[26] In brief, between November 2003 and February 2008, a total of 2644 women who fulfilled the inclusion criteria (≥16 years of age, intention to deliver at the reference hospital, no problems of communication, singleton pregnancy, and no assisted conception) were recruited during the first prenatal visit in 4 areas of study: Valencia (39°N latitude, n = 855), Sabadell (41°N latitude, n = 657), Gipuzkoa (42°N latitude, n = 638), and Asturias (43°N latitude, n = 494). Overall, 2505 (97%) women were followed until child's birth. Circulating 25(OH)D_3 concentrations in pregnancy were determined in 2389 women, and, among them, neuropsychological assessment was performed in 2112 (89% of eligible) infants. Exclusion criteria were birth at <37 weeks of gestation (n = 82), unknown gestational age (n = 16), underlying pathology (n = 17), poor-quality neuropsychological assessment (n = 124), and missing information on potential covariates (n = 53). Ultimately, 1820 (86% of eligible) mother-infant pairs were analyzed (Fig 1). The study was approved by the ethical committees of the centers involved in the study, and written informed consent was obtained from the parents of all children.

Assessment of Maternal Circulating 25(OH)D_3 Concentrations

A single maternal blood specimen was drawn during pregnancy (mean, 13.5 ± 2.1 weeks of gestation). Most blood draws (88%) were done during the second trimester of pregnancy (from 12 to 23 weeks of gestation), with 11.6% during the first trimester (<12 weeks of gestation), and <1% late in pregnancy (from 24 to 36 weeks of gestation). Samples were processed immediately and stored from −70 to −80°C until analysis. Maternal plasma concentrations of 25(OH)D_3 were quantified by high-performance liquid chromatography method by using a BioRAD kit according to Clinical and Laboratory Standard Institute protocols.[27] Detection limit was 5 ng/mL, and interassay coefficient of variation was 4.5%. The assay was validated by German Programmes of External Evaluation of Quality (DGKL-RFB-Referencezinstutuk fur Bionalytik), and results were satisfactory in 100% of the cases.

Assessment of Infant Neuropsychological Development

Neuropsychological development was assessed at age 14 months (range, 11–23 months) by using the Bayley Scales of Infant Development that assess age-appropriate mental (163 items) and psychomotor (81 items) development.[28] All testing was done by specially trained psychologists. Interobserver differences were quantified, and 3 sets of quality

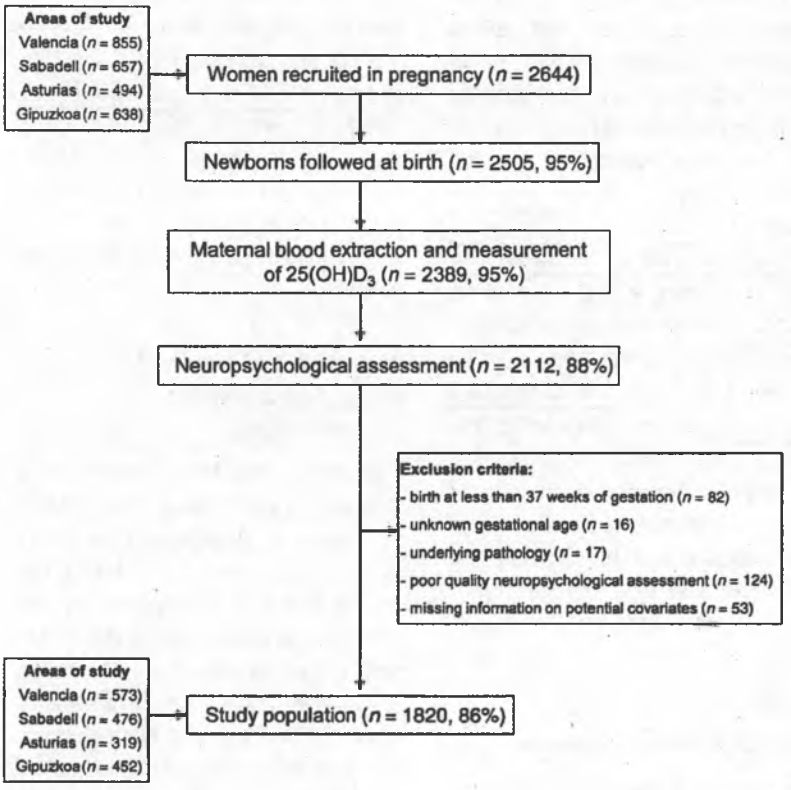

FIGURE 1
Flowchart of study population.

controls were undertaken. The inter-rater reliability, estimated by intraclass correlation, was 0.90 for mental scale and 0.96 for psychomotor scale. Furthermore, internal consistency determined by the Cronbach α-coefficient was 0.70 for mental scale and 0.73 for psychomotor scale. Raw scores were standardized for child's age in days at testing by using a parametric method for the estimation of age-specific reference intervals.[29] Normal distribution was adopted as starting point for model building. The parameters of the distribution (M and S curves, for the mean and SD, respectively) were modeled as a fractional polynomial (FP) function of age. FP models for the M and S curves were found by grid search of the powers in P by using Generalized Least Squares. The powers were selected from a small predefined set $P = \{-3, -2, -1, -1/2, 0 \text{ (log)}, 1/2, 1, 2, 3\}$ allowing up to FP with degree 3. Once the best powers in FP models have been chosen, the regression coefficients were estimated by maximum likelihood. Residuals were then standardized to a mean of 100 points with a SD of 15 points to homogenize the scales.

Potential Confounders

Based on previous knowledge, the following were considered a priori potential confounding factors because of their possible associations with maternal circulating 25(OH)D_3 concentration and neuropsychological test scores: child's gender, birth weight, maternal age, parity, maternal country of origin, parental social class, maternal education level, maternal pre-pregnancy BMI, and maternal smoking and alcohol consumption during pregnancy. Questionnaires during the first trimester of pregnancy obtained information about parity (0 vs 1 or more), maternal age, maternal country of birth (Spanish versus foreign), parental social class (defined as maternal or paternal occupation during pregnancy based on the highest social class by using a widely used Spanish adaptation of the international ISCO88 coding system) (I–II, managers/technicians; III, skilled; IV–V, semiskilled/unskilled), maternal education level (primary or less, secondary, university degree), and maternal pre-pregnancy BMI based on measured height at recruitment and pre-pregnancy self-reported weight (normal weight/underweight [≤24.99], overweight [25–29.99], obese [≥30]). Information on maternal smoking (no versus yes) and alcohol consumption during pregnancy (no versus yes, defined as consumption of alcohol beverages at least 1 time/month) was collected through questionnaires during the third trimester. All questionnaires were administered face-to-face by trained interviewers. Information related to child's gender, birth weight, and gestational age was obtained from clinical records.

Statistical Analysis

Maternal circulating 25(OH)D_3 concentrations were normally distributed. Clinically defined 25(OH)D_3 cut points were used: <20 ng/mL (reference group), 20 to 30 ng/mL, and >30 ng/mL.[30] Differences in baseline characteristics of participants across categories of maternal 25(OH)D_3 concentrations were compared by using χ^2 tests for categorical variables, analysis of variance for continuous variables with normal distribution, and Kruskal-Wallis tests for variables with skewed distributions. To adjust for month at blood collection, 2 approaches were used. In the first approach, we used "deseasonalization" of 25(OH)D_3 concentrations. In this approach seasonality of 25(OH)D_3 was tested by fitting the data to a sine function with a period of 12 months in a nonlinear regression cosinor model.[31] Then, the predicted 25(OH)D_3 concentrations based on month at

blood collection for each subject, derived from the sinusoidal model, were subtracted from the actual observed value. Subsequently, the overall mean was added and the resulting deseasonalized 25(OH)D_3 concentrations were analyzed. In the second approach, we used raw 25(OH)D_3 concentrations for the regression analysis in which we adjusted for month at blood collection. The results of these 2 approaches were essentially the same. Thus, the results using deseasonalized 25(OH)D_3 concentrations are presented in the main article, and the results using raw 25(OH)D_3 concentrations and adjusting for month are presented in the supplemental material (Supplemental Table 3). Linear dose-response relationship between maternal 25(OH)D_3 concentrations during pregnancy and infant neuropsychological development scores was assessed by using adjusted generalized additive models by graphical examination and likelihood ratio.[32] To examine the relationship between infant neuropsychological development and maternal 25(OH)D_3 concentrations, we used multivariable linear regression models. We treated circulating concentrations of 25(OH)D_3 as continuous (effect per 10 ng/mL) and clinically defined categories. Analyses were first adjusted for area of study (base model). Next, a fully adjusted model included child's gender, birth weight, maternal country of origin, maternal age, parental social class, maternal education level, parity, maternal pre-pregnancy BMI, and maternal smoking and alcohol consumption in pregnancy. There was no evidence that the associations of maternal 25(OH)D_3 concentrations with infant's neuropsychological scores differed between genders (all P values for interaction >0.2), and all results are presented for both genders combined. To preclude potential residual confounding, we assessed whether the associations were consistent across strata defined by maternal pre-pregnancy BMI, parental social class, and maternal education level. Analyses were conducted by using Stata software, version 11.1 (StataCorp, College Station, TX) and R statistical package version 2.13.0.

RESULTS

The study population included 49.7% male children and 58% firstborn. Ninety-two percent of mothers were born in Spain, and maternal mean age at child birth was 31.9 (4.2) years. Twenty-two percent of mothers had a low educational level (primary or less) and 33% were from high social class. Twenty-six percent of women were overweight/obese before pregnancy. Sixteen percent of women reported tobacco smoking and 19.6% alcohol consumption during pregnancy. Compared with excluded participants, those who were included in the present analysis showed higher birth weight, had higher social class, and mothers tended to smoke less during pregnancy, but did not differ in other main baseline characteristics (Supplemental Table 4).

The median plasma value of 25(OH)D_3 in pregnancy was 29.6 ng/mL (interquartile range, 21.8–37.3). A total of 356 (19.5%) pregnant women had vitamin D deficiency [25(OH)D_3 concentration <20 ng/mL], and 574 (31.5%) had vitamin D insufficiency [25(OH)D_3 concentration 20–30 ng/mL]. The characteristics of participants according to clinically defined cutoff points of circulating 25(OH)D_3 concentrations during pregnancy are shown in Table 1. Concentrations of circulating 25(OH)D_3 in pregnancy differed among areas of study, with Valencia area showing the highest concentrations and Asturias the lowest concentrations. Increasing trends across the clinically defined 25(OH)D_3 categories were observed for maternal age, parity, and maternal alcohol consumption. Decreasing trends across the categories of 25(OH)D_3 were found for lower parental social class and education level,

TABLE 1 Characteristics of Participants According to Maternal Circulating 25(OH)D_3 Concentrations in Pregnancy

	Serum 25(OH)D_3 Concentration			*P* Value Trend
	<20 ng/mL (n = 356)	20–30 ng/mL (n = 574)	>30 ng/mL (n = 890)	
Area of study				
Valencia (39°N latitude)	20.2	29.6	37.2	<.001
Sabadell (41°N latitude)	30.3	23.2	26.4	
Gipuzkoa (42°N latitude)	24.7	27.2	23.4	
Asturias (43°N latitude)	24.7	20.0	13.0	
Child's gender (male)	51.1	50.2	48.8	.722
Birth weight, g	3300 (435)	3283 (419)	3302 (419)	.685
Maternal age at child's birth, y	31.5 (4.5)	31.6 (4.1)	32.2 (4.0)	.004
Parity (1 or more)	36.8	41.6	44.3	.053
Maternal country of birth (non-Spanish)	9.0	6.8	7.4	.460
Parental social class				
I/II Managers/technicians	28.4	30.7	36.0	.037
III Skilled manual/nonmanual	26.1	27.5	26.5	
IV/V Semiskilled/unskilled	45.5	41.8	37.5	
Maternal education level				
Primary or less	23.9	22.1	22.3	.273
Secondary	43.5	43.6	39.4	
University degree	32.6	34.3	38.3	
Maternal pre-pregnancy BMI				
Normal weight/underweight (<24.99)	68.3	74.0	75.6	.086
Overweight (25–29.99)	22.5	19.0	16.6	
Obese (≥30)	9.3	7.0	7.8	
Smoking at third trimester (yes)	20.2	16.4	13.9	.022
Alcohol during pregnancy (yes)	18.0	16.4	22.4	.013

Values are percentages for categorical variables and mean (SD) for continuous variables.

among smokers and overweight mothers.

Maternal plasma concentrations of 25(OH)D_3 showed a seasonal distribution ($P < .05$, Fig 2). Maximum fitted concentrations of maternal plasma 25(OH)D_3 were observed in blood samples collected in August, and concentrations reached their nadir in February (Fig 2).

A positive linear relationship was found between circulating concentrations of maternal 25(OH)D_3 in pregnancy and both mental (Fig 3A) and psychomotor (Fig 3B) development scores in the offspring. In multivariable models, each 10 ng/mL increase in 25(OH)D_3 in pregnancy resulted in up to 0.79 and 0.88 points increase in mental and psychomotor development scores in offspring, respectively (Table 2). In the basic model with adjustment for area of study, infants of mothers with 25(OH)D_3 concentrations >30 ng/mL showed an advantage of 3.17 and 2.42 points in the mental and psychomotor scores, respectively, in comparison with those of mothers with 25(OH)D_3 concentrations <20 ng/mL (model 1) (Table 2). Although attenuated, these associations remained significant after adjustment for potential confounders including child's gender, birth weight, maternal country of origin, maternal age, parental socioeconomic status, maternal education level, parity, maternal pre-pregnancy BMI, and maternal smoking and alcohol consumption in pregnancy (model 2) (Table 2).

In addition, the associations did not differ according to maternal pre-pregnancy BMI, maternal social class or education level (all P values for interactions >0.1).

DISCUSSION

To our knowledge this is one of the first large-scale prospective pregnancy cohort studies to examine the association between maternal circulating 25(OH)D_3 concentrations in pregnancy and offspring neuropsychological development in infancy. Higher concentrations of circulating 25(OH)D_3 in pregnancy were associated with improved mental and psychomotor scores. Infants of mothers with 25(OH)D_3 concentrations >30 ng/mL (clinically considered as optimal levels) showed an advantage of 2.6 and 2.3 points in mental and psychomotor scores, respectively, in comparison with those of mothers with 25(OH)D_3 concentrations <20 ng/mL (considered as deficient levels). The association remained significant after adjusting for a wide range of potential confounding and intermediate factors.

The main strengths of this study include its population-based prospective design and large sample size as well as examination of the associations with plasma measurements of 25(OH)D_3 concentration, a reliable indicator of vitamin D status that also quantifies the outdoor exposure, rather than dietary reports that are likely to be influenced by reporting bias. Possible confounding was addressed in multivariable analyses adjusted for a wide range of potential confounding factors. Finally, we found a strong positive linear relationship at lower concentrations of maternal circulating 25(OH)D_3 (below 50–60 ng/mL), which supports the robustness of the findings; however, the generalization of this assumption at higher concentrations is limited owing to the small number of observations (ie, "sparse data bias").

The study has some limitations. First, only a single 25(OH)D_3 measurement per subject was available that could not reflect maternal long-term status during the entire pregnancy. Dealing with misclassification of estimated long-term vitamin D exposure by season of blood draw was accounted estimating deseasonalized 25(OH)D_3 concentrations based on a sinusoidal model. Second, we did not assess the effect of circulating 25(OH)D_2 concentrations, but, normally, majority of the 25(OH)D is in D_3 form. Third, the lack of information of infant's vitamin D status is another limitation. Fourth, we could not measure 25(OH)D_3 concentrations in all recruited subjects, which made selection bias possible. Participants were more likely to be female and parents had higher social class and education level; however, there was no evidence that the association between circulating maternal 25(OH)D_3 concentrations in pregnancy and infant's neuropsychological scores differed between genders, parental social class, or maternal education level. Fifth, parental intelligence, an important determinant of infant mental development, was not evaluated. However, parental education level and social class did not confound or modify

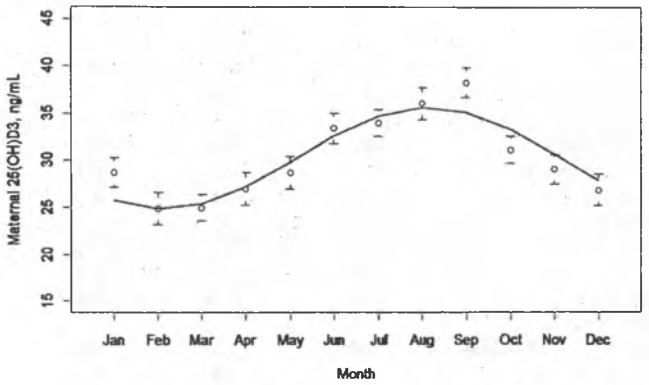

FIGURE 2
Fitted sinusoidal model for observed maternal circulating concentration of 25(OH)D_3 superimposed on plot of observed monthly mean and 95% CI values for 25(OH)D_3 concentration among 2112 participants in the INMA Project.

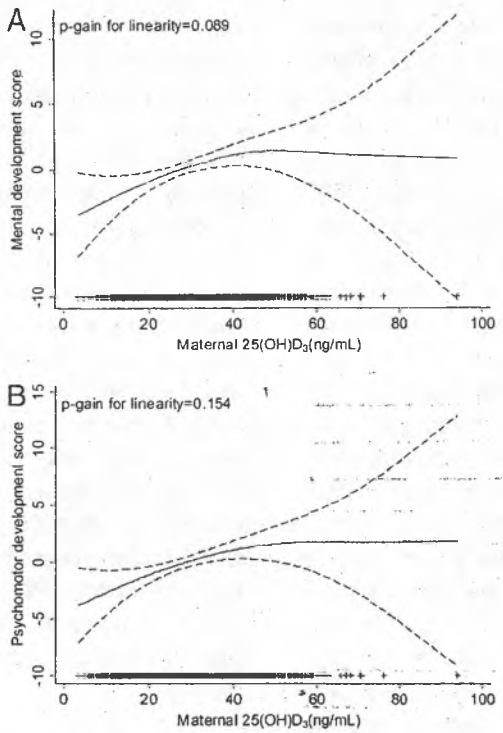

FIGURE 3

The relation (and 95% CI) of maternal circulating concentration of 25(OH)D_3 in pregnancy* (ng/mL) with mental (A) and psychomotor (B) developmental scores in infants. *Deseasonalized maternal 25(OHD)D_3 concentrations based on month at blood collection for each subject derived from the sinusoidal model. General additive models adjusted for area of study, child's gender, birth weight, maternal country of origin, maternal age, parental social class, maternal education level, parity, maternal pre-pregnancy BMI, and maternal smoking and alcohol consumption in pregnancy. The symbols (+) on the x axis indicate 25(OHD)D_3 observations.

TABLE 2 Association Between Maternal Circulating 25(OH)D_3 Concentrations[a] in Pregnancy and Neuropsychological Scores in Infants (n = 1820)

	Model 1[b]		Model 2[c]	
	β	(95% CI)	β	(95% CI)
Mental development score				
Continuous variable (per 10 ng/mL)	0.99	(0.33 to 1.65)	0.79	(0.14 to 1.45)
Categories (ng/mL)				
<20	Ref.		Ref.	
20–30	2.14	(0.08 to 4.19)	1.89	(−0.14 to 3.91)
>30	3.17	(1.19 to 5.16)	2.60	(0.63 to 4.56)
Psychomotor development score				
Continuous variable (per 10 ng/mL)	0.94	(0.29 to 1.60)	0.88	(0.22 to 1.54)
Categories (ng/mL)				
<20	Ref.		Ref.	
20–30	0.80	(−1.21 to 2.83)	0.86	(−1.16 to 2.88)
>30	2.42	(0.47 to 4.37)	2.32	(0.36 to 4.28)

[a] Deseasonalized maternal 25(OHD)D_3 concentrations based on month at blood collection for each subject derived from the sinusoidal model.

[b] Adjusted for area of study.

[c] Adjusted for area of study, child's gender, birth weight, maternal country of origin, maternal age, parental social class, maternal education level, parity, maternal pre-pregnancy BMI, and maternal smoking and alcohol consumption in pregnancy.

the associations, but their inclusion in the model cannot completely eliminate possible residual confounding by parental intelligence. Finally, we did not account for maternal physical activity and outdoor exposures (indicators of maternal fitness), which may result in some residual cofounding.

Two previous prospective studies have assessed the effect of maternal serum 25(OH)D_3 concentrations in pregnancy on offspring neurodevelopment.[19,20] The first study based on 178 mother-child pairs reported no association between circulating maternal 25(OH)D_3 concentrations, measured at 32 weeks of pregnancy, and offspring cognition performance at age 9 years.[19] However, in accordance with our results Whitehouse et al[20] have recently reported insufficient maternal serum 25(OH)D_3 concentrations, measured at 18 weeks pregnancy, to be associated with offspring language impairment at 5 and 10 years of age in a prospective study on 743 mother-child pairs. Lack of power in the Gale et al[19] study and differences in timing measurements (exposure and outcome) could explain controversial results between studies. Measurement of 25(OH)D_3 concentrations in Gale et al[19] study was performed in late pregnancy (median 32 weeks), whereas Whitehouse et al study and the current study determined 25(OH)D_3 concentrations earlier in pregnancy (mean, 18 and 13 weeks of gestation, respectively). Our results suggest that optimal concentrations of circulating 25(OH)D_3 as early as 13 weeks of gestation may have an impact on neuropsychological development in infancy, and vulnerability of the developing brain to vitamin D deficiency may be manifested early in pregnancy. The similarity in the effect magnitude of vitamin D deficiency in pregnancy on mental and psychomotor skills points out that different brain areas are affected and strengthen the idea that effects may occur during early development when brain structures begin to form and are thus vulnerable to damaging influences.[24] Furthermore, deficiencies in neuromotor development are associated with processes occurring early in fetal life.[33,34]

Studies assessing vitamin D status on cognitive functioning beyond childhood are scarce. A large population-based cross-sectional study conducted on 1676 adolescents aged 12 to 17 years has reported null association of different domains of cognitive function with 25 $(OH)D_3$ concentrations.[21,22] However, the cross-sectional design and the different timing at outcome assessment make the comparison with our study difficult.

Nevertheless, our results are consistent with previous studies conducted in elderly adults. Seven cross-sectional studies have reported lower concentrations of circulating $25(OH)D_3$ to be associated with higher risk of cognitive impairment.[9–11,13–15,18] Moreover, 3 cohort studies conducted on adults 65 years of age or older have also reported a trend for an independent association between lower circulating 25 $(OH)D_3$ concentrations and odds of cognitive decline.[12,16,17] Similar effects of vitamin D status on brain development and aging could indicate a higher susceptibility of the brain to vitamin D deficiency during these lifetime periods.

The biological basis for the association of vitamin D with cognitive function comes from evidence of the ubiquitous presence of vitamin D receptors and 1,α-hydroxylase (the terminal rate-limiting enzyme in the synthesis of calcitriol) in the rodent fetal and adult brain[35] and in the adult human brain.[36] Moreover, $25(OH)D_3$ crosses the blood-brain barrier and it is also synthesized in the brain.[36] Developmental vitamin D deficiency has been shown to modify the expression of multiple genes and proteins in brain tissue from offspring including neurotrophic factors and mitochondrial, cytoskeletal, and synaptic proteins.[8,37] In humans, there is some limited evidence for a relationship between vitamin D inadequacy and diverse neuropsychiatric conditions including multiple sclerosis,[38] depression,[39] schizophrenia,[40,41] and Alzheimer disease.[42] However, the evidence derived from animal studies strongly suggests a crucial role of vitamin D in brain development and critical brain functions,[2,3] normal neurogenesis,[7] learning ability and behavior.[43–45]

CONCLUSIONS

In this prospective cohort higher circulating $25(OH)D_3$ in pregnancy was associated with improved mental and psychomotor development in infants. Efforts to maintain an adequate vitamin D status in pregnancy could make a positive impact on infants' neuropsychological development if the associations are causal. Moreover, given the magnitude of vitamin D deficiency worldwide among pregnant women, the present results have important public health implications, and population-level consequences of vitamin D deficiency in pregnancy on brain development may be more profound in settings with higher prevalence of vitamin D deficiency. Additional studies are warranted to assess long-term effects of maternal vitamin D status in pregnancy on neuropsychological development in offspring.

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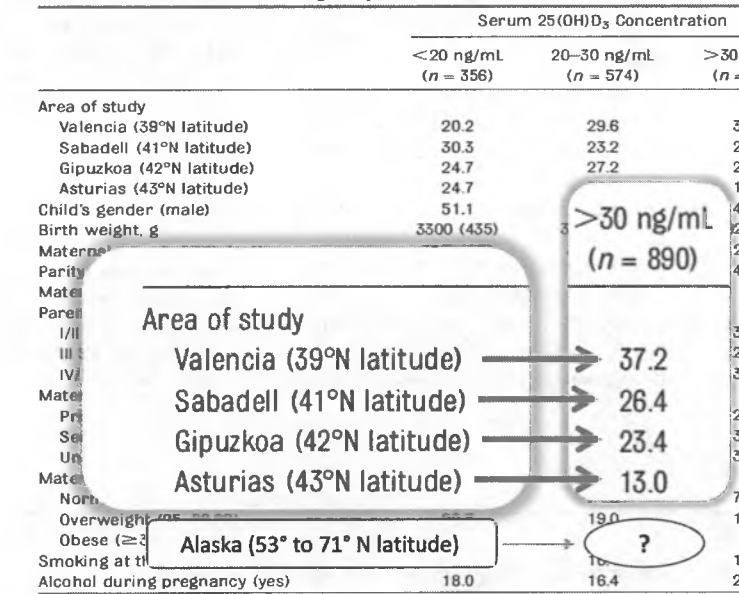
TABLE 1 Characteristics of Participants According to Maternal Circulating $25(OH)D_3$ Concentrations in Pregnancy

	Serum $25(OH)D_3$ Concentration			*P* Value Trend
	<20 ng/mL (*n* = 356)	20–30 ng/mL (*n* = 574)	>30 ng/mL (*n* = 890)	
Area of study				
Valencia (39°N latitude)	20.2	29.6	37.2	<.001
Sabadell (41°N latitude)	30.3	23.2	26.4	
Gipuzkoa (42°N latitude)	24.7	27.2	23.4	
Asturias (43°N latitude)	24.7	20.0	13.0	
Child's gender (male)	51.1	50.2	48.8	.722
Birth weight, g	3300 (435)	3283 (419)	3302 (419)	.685
Maternal age at child's birth, y	31.5 (4.5)	31.6 (4.1)	32.2 (4.0)	.004
Parity (1 or more)	36.8	41.6	44.3	.053
Maternal country of birth (non-Spanish)	9.0	6.8	7.4	.460
Parental social class				
I/II Managers/technicians	28.4	30.7	36.0	.037
III Skilled manual/nonmanual	26.1	27.5	26.5	
IV/V Semiskilled/unskilled	45.5	41.8	37.5	
Maternal education level				
Primary or less	23.9	22.1	22.3	.273
Secondary	43.5	43.6	39.4	
University degree	32.6	34.3	38.3	
Maternal pre-pregnancy BMI				
Normal weight/underweight (<24.99)	68.3	74.0	75.6	.086
Overweight (25–29.99)	22.5	19.0	16.6	
Obese (≥30)	9.3	7.0	7.8	
Smoking at third trimester (yes)	20.2	16.4	13.9	.022
Alcohol during pregnancy (yes)	18.0	16.4	22.4	.013

Values are percentages for categorical variables and mean (SD) for continuous variables.

TABLE 1 Characteristics of Participants According to Maternal Circulating $25(OH)D_3$ Concentrations in Pregnancy



Values are percentages for categorical variables and mean (SD) for continuous variables.

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High Prevalence of Vitamin D Insufficiency in Black and White Pregnant Women Residing in the Northern United States and Their Neonates[1]

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Abstract

In utero or early-life vitamin D deficiency is associated with skeletal problems, type 1 diabetes, and schizophrenia, but the prevalence of vitamin D deficiency in U.S. pregnant women is unexplored. We sought to assess vitamin D status of pregnant women and their neonates residing in Pittsburgh by race and season. Serum 25-hydroxyvitamin D (25(OH)D) was measured at 4–21 wk gestation and predelivery in 200 white and 200 black pregnant women and in cord blood of their neonates. Over 90% of women used prenatal vitamins. Women and neonates were classified as vitamin D deficient [25(OH)D <37.5 nmol/L], insufficient [25(OH)D 37.5–80 nmol/L], or sufficient [25(OH)D > 80 nmol/L]. At delivery, vitamin D deficiency and insufficiency occurred in 29.2% and 54.1% of black women and 45.6% and 46.8% black neonates, respectively. Five percent and 42.1% of white women and 9.7% and 56.4% of white neonates were vitamin D deficient and insufficient, respectively. Results were similar at <22 wk gestation. After adjustment for prepregnancy BMI and periconceptional multivitamin use, black women had a smaller mean increase in maternal 25(OH)D compared with white women from winter to summer (16.0 ± 3.3 nmol/L vs. 23.2 ± 3.7 nmol/L) and from spring to summer (13.2 ± 3.0 nmol/L vs. 27.6 ± 4.7 nmol/L) ($P < 0.01$). These results suggest that black and white pregnant women and neonates residing in the northern US are at high risk of vitamin D insufficiency, even when mothers are compliant with prenatal vitamins. Higher-dose supplementation is needed to improve maternal and neonatal vitamin D nutriture. J. Nutr. 137: 447–452, 2007.

Babies at birth deficient + insufficient = 92.4%
= 66.1%

Introduction

Rickets, once thought to have been nearly eradicated in the United States in the 1930s (1), has again become a major public health problem. Several reports have been published describing recent cases of rickets in infants, most of whom were black and exclusively breastfed (2–5). The reemergence of rickets is thought to be due to an epidemic of vitamin D deficiency in mothers and children (6). A newborn's vitamin D stores are completely reliant on vitamin D from the mother (7). Not surprisingly, poor maternal vitamin D status during pregnancy is a major risk factor for infant rickets (8–10).

In addition to causing poor global mineralization of the skeleton, vitamin D deficiency has implications for numerous other nonskeletal health outcomes. In utero or early life vitamin D deficiency has been linked to an increased risk of type 1 diabetes (11), asthma (12), and schizophrenia (13,14). Fascinating new data also show that vitamin D regulates placental development and function (15), which suggests that maternal vitamin D status may be associated with adverse outcomes of pregnancy, such as miscarriage, preeclampsia, and preterm birth.

The most important source of vitamin D is the skin's synthesis of the vitamin from UV B solar radiation (16). Any process that reduces UV B photons from entering the epidermis will diminish cholecalciferol (vitamin D-3) production. The skin pigment melanin absorbs UV B photons and can reduce vitamin D-3 synthesis by >90% (17). Consequently, African Americans are at high risk of vitamin D deficiency. The most recent data from the National Health and Nutrition Examination Survey (1988–1994) indicated that vitamin D deficiency [25-hydroxyvitamin D [25(OH)D] ≤37.5 nmol/L] was prevalent in 42% of black childbearing-aged women and only 4% of white childbearing-aged women residing throughout the United States (18). Vitamin D status is also worsened in winter months (November through March), when, at latitudes above 37°, less UV B radiation reaches the earth and little or no vitamin D can be synthesized in the skin (16,19). Indeed, vitamin D deficiency in U.S. childbearing-aged women was more than 3 times as common in winter than summer in both blacks and whites (18).

Despite the striking racial disparity in vitamin D deficiency and the strong influence of season, there are few recent investigations into the vitamin D status of U.S. black and white pregnant women and their neonates throughout the year. Given

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the public health importance of adequate vitamin D in this group and the racial disparity in a number of relevant disease outcomes, we sought to assess the vitamin D status of a cohort of nulliparous pregnant women residing in Pittsburgh, Pennsylvania (latitude 40°N) and their neonates by race and season.

Subjects and Methods

Data came from the Pregnancy Exposures and Preeclampsia Prevention Study (20). Women carrying singleton fetuses were enrolled at <16 wk gestation from outpatient clinics at Magee-Women's Hospital in Pittsburgh, Pennsylvania and affiliated private practices from 1997 to 2001. The response rate was 72%. After providing informed, written consent, all subjects completed an interviewer-administered questionnaire at enrollment to collect data on sociodemographic factors, medical history, and health behaviors. Nonfasting blood samples were collected at times of usual blood draws for clinical indications (initial visit, 16–18 wk, 26–29 wk, and predelivery) and banked. Medical records were abstracted to ascertain blood pressures and urinary protein measurements throughout gestation, delivery data, and neonatal outcomes. Venous cord serum samples were also collected and banked. The University of Pittsburgh and Magee-Women's Hospital Institutional Review Boards approved the study.

A total of 2211 women enrolled in the study and had complete data on pregnancy outcomes. For the current analysis, we used SAS random number generator to randomly select 200 white women and 200 black women who were nulliparous (i.e. had no previous pregnancies lasting >20 wk), had no preexisting medical conditions, and had an uncomplicated index pregnancy delivered at term (37–42 wk) with an appropriately grown infant. From each of the 400 women, we sought to select 1 serum sample at <22 wk gestation, 1 predelivery serum sample, and a cord serum sample. If a woman had more than 1 sample collected at <22 wk gestation, we randomly selected 1 sample using SAS random number generator. Of the 200 white women selected, 198 had an available banked serum sample collected at <22 wk, 199 had an available banked serum sample collected predelivery, and 195 had an available banked cord serum sample. These numbers were 194, 185, and 171 for the 200 black women, respectively.

Quantitation of serum 25(OH)D. Maternal and cord serum samples were stored in aliquots at −80°C until they were analyzed for 25(OH)D. Quantitation of serum 25(OH)D was performed using a commercial ELISA from Immunodiagnostic Systems Limited and validated against an HPLC method. The ELISA was performed according to the manufacturer's written protocol and all samples were assayed in duplicate. The ELISA could detect 25(OH)D in the range of 5–300 nmol/L. No sample in our analysis fell outside this detectable range. The interassay CV for the ELISA was 10.3%. The ELISA recognized 100% of 25(OH)D3 and 75% of 25(OH) ergocalciferol (vitamin D-2) but did not distinguish between these 2 forms. In our initial HPLC validation, we observed that only 3 of 32 samples (<10%) had any measurable 25(OH)D2, and within these samples, 25(OH)D2 accounted for only 10% of the total measurable 25(OH)D.

The HPLC method for the quantification of 25(OH)D was modified from those published by Horst et al. (21) and Alvarez and De Mazancourt (22). Briefly, serum samples were spiked with an internal standard (1 α-hydroxy vitamin D-3 purchased from Sigma) to a final concentration of 50 nmol/L. Samples were processed twice by solid phase extraction, first using C_{18} Sep-pak cartridges and then using Silica Sep-pak cartridges. After extraction, samples were dried completely under a nitrogen stream at 37°C and reconstituted in 100% acetonitrile. Fifty microliters of the extracted samples was injected onto a prepared HPLC system that consisted of a Waters 600E pump, a Waters 717 plus autosampler, and a Waters 996 photodiode array. We separated samples on a C18 column (150 × 4.8 mm, 5μm, Supelco) with a 20- × 4.8-mm guard column in line. The HPLC method could separate 1 α-hydroxy vitamin D-3, 25(OH)D2, and 25(OH)D3 using isocratic conditions with a mobile phase that consisted of 87% acetonitrile with all components being detected at a wavelength of 265 nm. For most serum samples, only 25(OH)D3 was detectable. However, a few samples had small but measurable amounts of 25(OH)D2. The interassay CV for 25(OH)D3 using the HPLC method was 5.8%. The sensitivity of the HPLC method was <10 nmol/L and had a linear range to 1000 nmol/L. The relation between serum 25(OH)D concentrations obtained from the ELISA compared with HPLC was as follows: slope = 1.14, intercept = 22, $r = 0.88$. Because the ELISA overestimated concentrations of 25(OH)D by ~25%, we adjusted the values obtained by ELISA so they would be in better agreement with data obtained by HPLC.

For our analysis, we classified women and neonates into 1 of 3 groups that defined vitamin D status: vitamin D deficiency [25(OH)D <37.5 nmol/L], vitamin D insufficiency [25(OH)D 37.5–80 nmol/L], and vitamin D sufficiency [25(OH)D >80 nmol/L] (6,23). The same cutoffs were used for both women and neonates, because experts contend that there is no reason to think the definition of vitamin D sufficiency varies by age (6).

Race/ethnicity was self-reported as non-Hispanic white or non-Hispanic black. Season of sample collection was defined as winter (December, January, February), spring (March, April, May), summer (June, July, August), and fall (September, October, November). Prepregnancy BMI [weight (kg)/height $(m)^2$] was based on measured height and maternal self-report of prepregnancy weight at the initial visit. Self-reported data were available on gravidity (i.e. number of times a woman has been pregnant: 1, 2, ≥3), marital status (married, unmarried), maternal education (<12, 12, >12 y), and smoking status (smoker, nonsmoker). At enrollment, women self-reported their regular use of multivitamins or prenatal vitamins in the periconceptional period (defined as the 3 mo before and 3 mo after conception), and at delivery, women reported their regular use in the last 3 mo of pregnancy.

Statistical analysis. Nonparametric data were log-transformed before statistical tests were performed. Student's t tests and chi-square tests were used to compare mean 25(OH)D concentrations and proportion of vitamin D deficiency and insufficiency by race/ethnicity. A P-value of 0.05 defined significance. Spearman rank correlation coefficient was used to test for correlations between maternal and cord serum 25(OH)D concentrations. Multivariable regression models were built to assess the independent effect of season on vitamin D status of mothers and newborns. First, we fitted 2 multivariable linear regression models with either maternal or neonatal 25(OH)D concentration as the dependent variable and a series of indicator variables for season as the primary independent variables. Second, we fitted 2 multivariable log-binomial regression models with either maternal or neonatal vitamin D insufficiency (binary) as the dependent variable and season as the primary independent variable. We used generalized estimating equations for both maternal linear and log-binomial models to account for the nonindependence of repeated serum measurements among women (24).

We fit parsimonious regression models by specifying full models with potential effect modifiers and confounding variables (season, race/ethnicity, maternal age, education, marital status, gravidity, maternal prepregnancy BMI, smoking status, periconceptional multivitamin use, multivitamin use in the last 3 mo of pregnancy, and sample gestational age). Effect modification by race and blood sample gestational age were tested separately using Wald P-values ($\alpha = 0.10$). Potential confounders were considered to not be influential and were removed from the model if their inclusion did not satisfy our a priori change-in-estimate criterion (a change in the coefficient of >8%). Sample gestational age, maternal prepregnancy BMI, and periconceptional multivitamin use met our definition of confounding and were included in the final models.

Values in the text are means ± SEM or mean [95% CI]. Stata software version 8.0 was used for all data analysis.

Results

Black women were more likely than white women to be <20 y old, unmarried, less educated, nonsmokers, obese, and nonusers of periconceptional multivitamins (Table 1). The maternal serum samples were drawn at similar gestational ages in black and white women.

TABLE 1 Characteristics of white and black pregnant nulliparous women[1]

	White women, $n = 200$	Black women, $n = 200$	P-value
Maternal age, %			
<20 y	23.5	54.0	<0.01
20–29 y	56.5	41.0	
≥30 y	41.0	5.0	
Marital status, %			
Married	38.0	3.0	<0.01
Unmarried	62.0	97.0	
Maternal education, %			
<12 y	15.5	31.0	<0.01
12 y	25.0	33.0	
>12 y	59.5	36.0	
Gravidity,[2] %			
1	72.5	69.5	0.31
2	19.5	24.0	
≥3	8.0	6.5	
Smoking status, %			
Smokers	52.5	41.5	<0.05
Nonsmokers	47.5	58.5	
Prepregnancy BMI, %			
<18.5 kg/m^2	8.0	4.0	<0.01
18.5–24.9 kg/m^2	58.0	48.5	
25.0–29.9 kg/m^2	21.0	23.0	
≥30.0 kg/m^2	13.0	24.5	
Regular[3] periconceptional[4] multivitamin use, %			
Yes	66.5	33.5	<0.01
No	44.5	55.5	
Regular[3] multivitamin use in the last 3 mo of pregnancy, %			
Yes	94.3	91.9	0.36
No	5.7	8.1	
Median (range) gestational age of blood sample <22 wk	10.2 (4.4–20.9)	11.7 (4.7–20.4)	0.06
Median (range) gestational age of blood sample at delivery, *wk*	40.0 (37–42.3)	39.9 (37–42)	0.89

[1] Data are presented as percent or median (range).
[2] Defined as the number of times a woman has been pregnant.
[3] Defined as self-reported use of multivitamins or prenatal vitamins at least once per week.
[4] Defined as the 3 mo before conception and the 3 mo after conception.

TABLE 2 Vitamin D status of white and black pregnant women and their neonates[1]

	White women, $n = 200$	Black women, $n = 200$
4–21 wk gestation		
Serum 25(OH)D,[2] *nmol/L*	73.1 (69.4, 76.9)	40.2 (37.9, 42.7)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	2.0	44.9**
Insufficient: 25(OH)D 37.5–80 nmol/L	60.3	51.0
Sufficient: 25(OH)D >80 nmol/L	37.3	4.1
37–42 wk gestation		
Serum 25(OH)D, *nmol/L*	80.4 (76.0, 85.1)	49.4 (46.1, 52.9)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	5.0	29.2**
Insufficient: 25(OH)D 37.5–80 nmol/L	41.2	54.1
Sufficient: 25(OH)D >80 nmol/L	53.8	16.7
Cord blood		
Serum 25(OH)D, *nmol/L*	67.4 (63.8, 71.3)	39.0 (36.3, 41.8)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	9.7	45.6**
Insufficient: 25(OH)D 37.5–80 nmol/L	56.4	46.8
Sufficient: 25(OH)D >80 nmol/L	33.9	7.6

[1] Values are geometric means [95%CI] or %. *Different from white women, $P <$ 0.001 (student's t test); **different from white women, $P < 0.001$ (chi-square test).
[2] Log-transformed to ensure normality.

The unadjusted mean maternal serum 25(OH)D concentrations at 4–21 wk gestation and at term were significantly higher among white women than black women (Table 2). At 4–21 wk gestation, the vast majority of black women were either vitamin D deficient or insufficient. This is in sharp contrast to white women, almost none of whom were vitamin D deficient at 4–21 wk gestation. However, white women had a high likelihood of vitamin D insufficiency. Results were similar for black and white women in samples collected before delivery (Table 2). Notably, we observed differences in mean 25(OH)D at 4–21 wk by periconceptional multivitamin use. Regular users of periconceptional multivitamins had higher serum 25(OH)D than nonusers in both white [76.7 (72.0, 81.7) nmol/L vs. 68.8 (63.2, 74.9) nmol/L, $P < 0.05$] and black women [46.0 (42.0, 50.5) nmol/L vs. 37.7 (35.0, 40.6) nmol/L, $P < 0.01$].

The unadjusted mean cord serum 25(OH)D concentrations were significantly higher in offspring of white mothers than offspring of black mothers (Table 2). Among white neonates, about two-thirds had 25(OH)D ≤80 nmol/L. Furthermore, unadjusted mean cord serum 25(OH)D was lower in mothers who had a predelivery serum 25(OH)D ≤80 nmol/L than mothers with 25(OH)D >80 nmol/L [mean [95%CI]: black mothers: 34.2 (32.0, 36.7) nmol/L vs. 76.0 (68.6, 84.1) nmol/L, $P <$ 0.001; white mothers: 51.2 (47.6, 55.2) nmol/L vs. 86.2 (82.4, 90.1) nmol/L, $P < 0.001$]. Cord serum 25(OH)D concentrations had a moderate positive correlation with maternal serum 25(OH)D at 4–21 wk gestation ($r = 0.58$, $P < 0.001$) and a strong positive correlation with maternal serum 25(OH)D before delivery ($r =$ 0.89, $P < 0.001$).

In both racial/ethnic groups, maternal (Fig. 1*A*) and cord serum (Fig. 1*B*) 25(OH)D concentrations were highest in summer and lowest in winter and spring (Fig. 1*A*,*B*). However, the amplitude of the difference from winter to summer and spring to summer varied by race/ethnicity. After adjustment for sample gestational age, prepregnancy BMI, and multivitamin use, black women had a smaller mean difference in adjusted maternal 25(OH)D compared with white women from winter to summer (16.0 ± 3.3 nmol/L vs. 23.2 ± 3.7 nmol/L) and from spring to summer (13.2 ± 3.0 nmol/L vs. 27.6 ± 4.7 nmol/L). This race-by-season interaction was important ($P < 0.01$). Similarly, black newborns had a smaller mean adjusted cord blood 25(OH)D increase than white newborns from spring to summer (11.3 ± 6.1 nmol/L vs. 29.3 ± 4.8 nmol/L, $P < 0.05$). There were no significant differences in other seasonal contrasts among black and white neonates.

Vitamin D insufficiency in mothers and their neonates was most common in spring among whites and in winter among blacks (Fig. 2*A*–*C*). After adjustment for gestational age, prepregnancy BMI, and multivitamin use, we observed an interaction between race and season on the likelihood of vitamin D insufficiency [defined here as 25(OH)D ≤80 nmol/L] in maternal

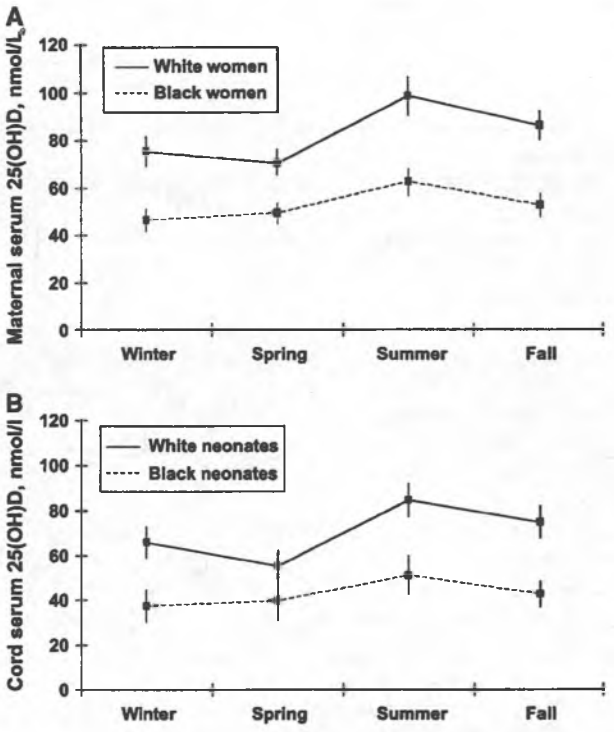

Figure 1 Maternal serum 25(OH)D concentrations by season in 200 white and 200 black pregnant women (*A*) and cord serum 25(OH)D concentrations of their neonates (*B*). All values were adjusted for prepregnancy BMI, prenatal vitamin use, and gestational age.

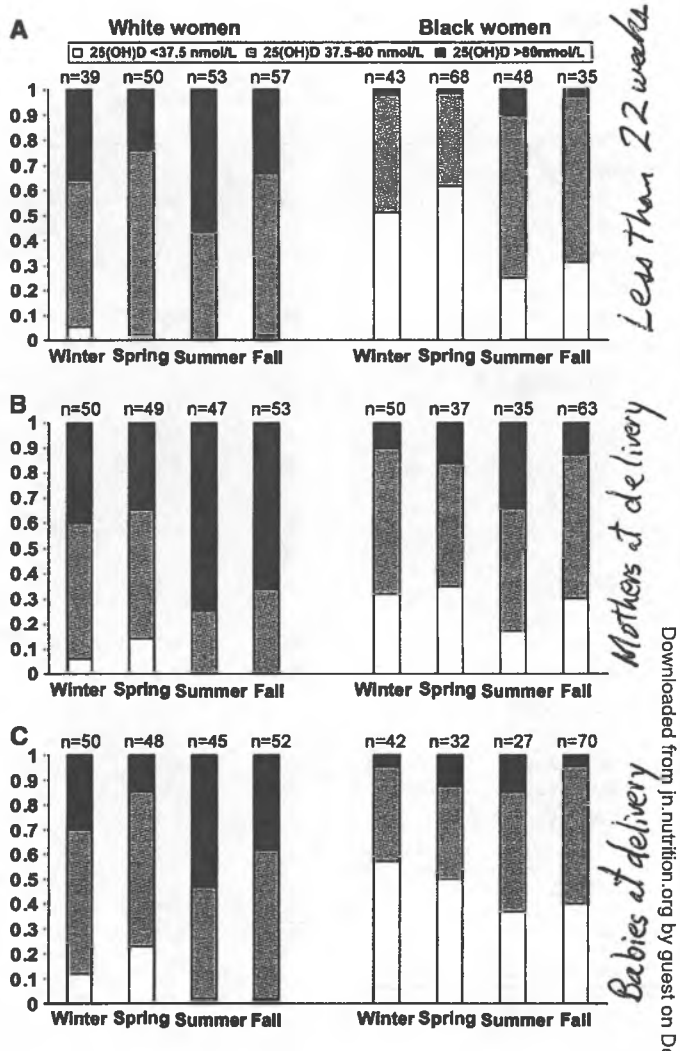

Figure 2 Prevalence of vitamin D deficiency [25(OH)D <37.5 nmol/L], insufficiency [25(OH)D 37.5–80 nmol/L], and sufficiency [25(OH)D >80 nmol/L] among 200 white and 200 black women at 4–21 wk gestation (*A*), at term (*B*), and in their neonates (*C*).

samples ($P < 0.001$). Compared with summer, the adjusted prevalence of vitamin D insufficiency was 1.9-fold, 2.1-fold, and 1.5-fold in winter, spring, and fall, respectively, among white mothers (Table 3). White neonates born in spring had a 76% increased prevalence of vitamin D insufficiency compared with white neonates born in summer. Among black women and newborns, the adjusted prevalence ratios were significantly attenuated compared with those seen in whites and did not show great seasonal variability. Indeed, compared with summer, there was only a 16–21% increase in the likelihood of vitamin D insufficiency in winter, spring, and fall among black women and no significant seasonal effect in black neonates (Table 3).

Discussion

In this cohort of black and white nulliparous pregnant women with a normal pregnancy outcome who resided in Pittsburgh (latitude 40°N), we found a remarkably high proportion of vitamin D deficiency and insufficiency among mothers throughout gestation and among their infants at birth. Although black women and newborns carried the burden of vitamin D insufficiency, a striking number of white women and their neonates were also affected. Notably, we observed low concentrations of 25(OH)D despite >90% of women reporting regular multivitamin use in the last trimester of pregnancy and 45% reporting regular multivitamin use in the periconceptional period.

Studies reporting a high prevalence of vitamin D deficiency among pregnant women and their neonates are abundant, particularly among Middle Eastern women (25–30), veiled or deeply pigmented Australians (31), non-Westerners in the Netherlands (32), Athenians (33), and South Asians living in Asia (34,35), the United Kingdom (36,37), and Europe (38,39). Yet, we are aware of only 2 studies that assessed 25(OH)D concentrations in black and white pregnant women in the United States, both of which were conducted over 20 y ago with small sample sizes. In a cross-sectional study of 10 black and 12 white pregnant women residing in Cleveland (latitude 41°N) in February and March, investigators reported results consistent with ours: plasma 25(OH)D at term was lower in black mothers (45.5 ± 30.3 nmol/L) than white mothers (68.5 ± 21.8 nmol/L, $P < 0.05$) and in black neonates (23.8 ± 16.8 nmol/L) than white neonates (37.3 ± 13.5 nmol/L, $P < 0.05$) (7). In contrast, another study in St. Louis (latitude 38°N) of 56 women in February and 61 women in August found that the mean 3rd-trimester serum 25(OH)D was significantly lower in February (38.5 ± 14.8 nmol/L) than August (105 ± 34.8 nmol/L) but that there were no differences between black and white women at either time (40). In a postpartum study of 40 mostly black mother-infant pairs, investigators reported that 50% of mothers had 25(OH)D <30 nmol/L at 24–48 h postpartum despite a majority of women using a multivitamin during pregnancy (41).

TABLE 3 Prevalence ratios (PR) for maternal vitamin D insufficiency [serum 25(OH)D ≤80 nmol/L] among white and black pregnant women and their offspring[1]

	White women, $n = 200$	Black women, $n = 200$
Maternal serum[2]		
Winter	1.90 (1.44, 2.49)	1.21 (1.07, 1.35)
Spring	2.08 (1.60, 2.70)	1.16 (1.04, 1.30)
Summer	1.00 (ref)	1.00 (ref)
Fall	1.50 (1.13, 2.00)	1.19 (1.05, 1.36)
Cord serum[3]		
Winter	1.55 (0.90, 2.66)	1.12 (0.67, 1.88)
Spring	1.76 (1.03, 3.00)	1.01 (0.57, 1.79)
Summer	1.0 (ref)	1.0 (ref)
Fall	1.30 (0.75, 2.27)	1.11 (0.69, 1.79)

[1] Values are adjusted PR (95% CI). Adjusted for gestational age of the sample, prepregnancy BMI, and multivitamin use.
[2] Results are based on a multivariable log-binomial regression model using generalized estimating equations to account for the nonindependence of repeated 25(OH)D measures in mothers across pregnancy.
[3] Results are based on a multivariable log-binomial regression model.

To our knowledge, ours is the first pregnancy study that used the 80-nmol/L cut-point to define vitamin D insufficiency. This cutoff is recommended because it correlates with a number of nutritional biomarkers that are impaired by inadequate vitamin D status (23). Previous investigators used more conservative cut-points, which underestimate the magnitude of the problem (23). If we had only reported 25(OH)D concentrations <37.5 nmol/L, nearly all of the white women identified as insufficient with the 80 nmol/L cutoff would have been missed.

Similar to other investigators (7,34,35,42), we found a strong correlation between maternal and cord blood 25(OH)D. Indeed, the high incidence of vitamin D insufficiency in mothers is additionally relevant, because newborns will also have inadequate vitamin D stores to draw on in early life. Although we lacked data on other functional indicators of vitamin D status, such as intact parathyroid hormone, we can assume that when 25(OH)D concentrations are ≤80 nmol/L, bone mineral density is compromised, and below 37.5 nmol/L, individuals are at risk of osteomalacia or rickets (6).

The seasonal changes that we observed in 25(OH)D concentrations are well recognized (19,43,44), but few investigators have examined how these seasonal patterns differ between blacks and whites. Our finding of an attenuated increase in mean 25(OH)D and the likelihood of vitamin D insufficiency from winter and spring to summer among black women and black neonates compared with their white counterparts is consistent with 2 studies conducted in U.S. childbearing-aged women (18,45) and 1 in New Zealand nonpregnant adults (46). These data support the observation that with typical sunlight exposure, black individuals synthesize less cutaneous vitamin D than do whites (47), leading to an inability of their vitamin D status to recover to adequate levels when sunlight increases in summertime.

Our study was limited by a lack of data on sunlight exposure, dietary vitamin D intake, and skin type/skin pigmentation. Such data would have allowed us to examine the extent to which important determinants of vitamin D status contribute to deficiency in pregnant women and newborns and how they vary by race and season. We also lacked data on the prenatal vitamin brand and dose used by our subjects. Such information would have allowed us to determine whether women were supplemented with vitamin D-2 or vitamin D-3 and the amount they received daily. As mentioned previously, we did not measure functional indicators of maternal or neonatal vitamin D status, such as bone density measurements. Nevertheless, our biracial cohort of 400 mostly low-income women residing at 40°N latitude allowed us to study patterns of 25(OH)D concentrations across seasons. Importantly, our use of the 80-nmol/L cut-point to define vitamin D insufficiency provided the most accurate description of vitamin D nutriture in pregnant women and neonates. Another major strength was the validation of our method of quantifying serum 25(OH)D against HPLC, the gold standard (48).

Our study suggests that black and white pregnant women residing in northern United States and their neonates are at high risk of vitamin D insufficiency, even when they regularly use a prenatal vitamin or multivitamin. Our data add to the growing body of research supporting assertions that the current vitamin D dietary intake recommendations are inadequate to meet the increased demands of pregnancy (16,23,49). Research into the true vitamin D requirements of pregnancy is greatly needed. With the mounting evidence that vitamin D insufficiency increases the risk of skeletal problems, autoimmune diseases, cancers, type 1 diabetes, heart disease, and schizophrenia (14,16), and the striking racial disparities in many of these health outcomes, improving maternal vitamin D status in pregnancy has a tremendous capacity to benefit public health.

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TABLE 2 Vitamin D status of white and black pregnant women and their neonates[1]

	White women, $n = 200$	Black women, $n = 200$
4–21 wk gestation		
Serum 25(OH)D,[2] *nmol/L*	73.1 (69.4, 76.9)	40.2 (37.9, 42.7)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	2.0	44.9**
Insufficient: 25(OH)D 37.5–80 nmol/L	60.3	51.0
Sufficient: 25(OH)D >80 nmol/L	37.3	4.1
37–42 wk gestation		
Serum 25(OH)D, *nmol/L*	80.4 (76.0, 85.1)	49.4 (46.1, 52.9)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	5.0	29.2**
Insufficient: 25(OH)D 37.5–80 nmol/L	41.2	54.1
Sufficient: 25(OH)D >80 nmol/L	53.8	16.7
Cord blood		
Serum 25(OH)D, *nmol/L*	67.4 (63.8, 71.3)	39.0 (36.3, 41.8)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	9.7	45.6**
Insufficient: 25(OH)D 37.5–80 nmol/L	56.4	46.8
Sufficient: 25(OH)D >80 nmol/L	33.9	7.6

[1] Values are geometric means [95%CI] or %. *Different from white women, $P < 0.001$ (student's t test); **different from white women, $P < 0.001$ (chi-square test).
[2] Log-transformed to ensure normality.

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Vitamin D status of exclusively breastfed infants aged 2-3 months.

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Abstract

BACKGROUND: New Zealand in 2008 adopted WHO policy which recommends that all infants are exclusively breast fed until 6 months of age. The benefits of this policy for the infant are undisputed; however, this policy has the potential to adversely impact on infant vitamin D status. A number of countries now recommend that all breastfed infants receive daily vitamin D supplementation of 400 IU to prevent rickets. New Zealand has no policy on the vitamin D supplementation of 'low-risk' breastfed infants. There are no data on the vitamin D status of exclusively breastfed infants in the first few months of life in New Zealand.

AIM: To describe serum 25-hydroxy-vitamin D (25(OH)D) concentrations in exclusively breastfed infants aged 2-3 months.

DESIGN/METHODS: Healthy term exclusively breastfed infants who were receiving no vitamin D supplements were enrolled over a 15-month period. A capillary blood sample was obtained from each infant. Serum 25(OH)D was measured using isotope-dilution liquid chromatography-tandem mass spectrometry.

RESULTS: 94 infants were enrolled (mean age 10 weeks). Median 25(OH)D concentration was 53 nmol/l (IQR 14-100 nmol/l). 23 (24%) infants had serum 25(OH)D concentration <27.5 nmol/l. Infants enrolled during winter had a median (IQR) 25(OH)D serum concentration of 21 nmol/l (14,31). Infants enrolled during summer had a median (IQR) 25(OH)D concentration of 75 nmol/l (55 100) (winter vs summer, $p<0.0001$).

CONCLUSIONS: Vitamin D deficiency is prevalent in exclusively breastfed infants in New Zealand. Vitamin D supplementation should be considered as part of New Zealand's child health policy.

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Vitamin D levels of infants converted from nmol/l to the US common measurement for blood serum concentrations, ng/ml:

-Entire study:
- Median = 21.2 ng/ml
- 24% of infants < 11 ng/ml

-Infants enrolled in winter:
- Median = 8.4 ng/ml
- Highest level = 12.4 ng/ml

-Infants enrolled in summer:
- Median = 30 ng/ml

Conversion notes by the office of Representative Seaton

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Chapter 5

VITAMIN D AND SUICIDE RISK FACTORS

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Abstract

Low vitamin D levels are negatively associated with certain prosuicidal factors such as exacerbation of depression, anxiety, psychosis, and certain medical conditions. Therefore, we hypothesize that they may also be associated with completed suicides. In particular, lower vitamin D levels at the end of winter, secondary to the lower vitamin D production in the skin, (as a result to reduced skin surface exposure as well as reduced duration of exposure, an after effect of uncomfortably low heat index and lower solar radiation). In preparation to test this hypothesis in future research, we now briefly review the existent literature on vitamin D, its deficiency and its reported association with certain risk factors for suicide.

Introduction

Suicide is the 10th leading cause of death worldwide and the second leading cause of death in adolescents and adults ages 15-35 years (1-3). Suicide attempts are 2 to 3 times more likely than fatal completions (4). Approximately 90% of individuals who die by suicide are diagnosable with a psychiatric illness. About 9.5% of the United States population suffers from a mood disorder including 6.7% suffering from major depressive disorder, 18.1% diagnosed with an anxiety disorder and 1.1% with a psychotic disorder expressed by

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schizophrenia (1-3,5). The risk of suicidal behavior markedly increases if an individual manifests co-morbidity. For instance, in a study performed on adolescents and young adults with suicide attempts, 79% of suicidal individuals had co-morbid psychiatric disorders, and individuals with 3 or more diagnoses of these disorders were significantly more likely to attempt suicide as compared to healthy controls (4, 6).

Vitamin D deficiency results from insufficient amounts of the circulating vitamin D, which is essential for proper bone and mineral metabolism, growth, neurodevelopment and immune maturation. A significant amount of vitamin D is synthesized in the skin under the influence of ultraviolet light from the sun.

Many individuals reside in areas of the world with limited sunlight exposure, such as cold climate and higher latitudes. Other implications include clothing choices that tend to cover the skin while outside, or not eating enough foods rich in vitamin D, such as fatty fish or dairy products. These, if not adequately corrected with vitamin supplements, could lead to vitamin D deficiency.

The objective of this chapter is to evaluate evidence suggesting an association between vitamin D serum levels and suicide risk factors, such as 1) Psychiatric Disorders, including anxiety, mood and psychotic disorders, 2) family history of suicide, including genetic and early developmental factors and 3) chronic medical illnesses. We will also discuss Vitamin D physiology and the possibility of its use as a preventive measure for suicidal behaviors.

Figure 1. Vitamin D deficiency, and suicide predispositions and triggers such as negative life events, if combined, can lead to suicide.

What Is Vitamin D?

Vitamin D is a group of fat-soluble prohormones, the two forms of which are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). The difference between these two forms lies in their side chain. Vitamin D_3 is either formed in the skin after exposure to ultraviolet light (natural sunlight or artificial) or it is obtained orally from dietary sources. Natural, enriched and supplemental sources of vitamin D are shown in Table 1.

7-dehydrocholesterol (pre-vitamin D_3) is the derivative of cholesterol and is formed in skin under the influence of ultraviolet (UV) light. Vitamin D_2 is obtained by irradiation of plant materials or foods. The highest concentrations of 7-dehydrocholesterol are present in epidermal layers of the skin. This pre-vitamin D_3 is then spontaneously isomerized to vitamin D_3 in the skin.

Table 1. Sources of vitamin D

NATURAL, ENRICHED AND SUPPLEMENTAL SOURCES OF VITAMIN D	
Nutritional Sources	Vitamin D Content
NATURAL	
Salmon	
Fresh (3.5 oz)	600-1000 IU
Farmed (3.5 oz)	100-250 IU
Canned Tuna (3.6 oz)	250 IU
Shiitake Mushrooms	
Fresh (3.5 oz)	100 IU
Sun-Dried (3.5 oz)	1,600 IU
Yolk of an egg	20 IU
ENRICHED	
Milk (8 oz)	100 IU
Orange Juice (8 oz)	100 IU
Cereals (1 serving portion)	100 IU
SUPPLEMENTS	
Infant formula (8 oz)	100 IU
Ergocalciferol	50,000 IU/Capsule
Dristol liquid supplement	8000 IU/Ml
Over-Counter Multivitamin	400 IU
UV-B Radiation (5-10 min in sunlight)	3,000 IU

This Vitamin D_3 formed in the skin, where it can meet one of two fates. It can be converted into active vitamin D_3 ($1,25[OH]_2D_3$) (calcitriol) within the skin or it can be transported to the liver after binding with proteins in the blood. Both vitamin D_2 and D_3 undergo the same activation process involving first, 25-hydroxylation in the liver, followed by 1 alpha-hydroxylation in the kidney to make the biologically active compounds $1,25[OH]_2D_2$ and $1,25[OH]_2D_3$, respectively. There is little evidence that these two active forms differ in their mode of action, and since most is known about the synthesis and action of $1,25[OH]_2D_3$, most studies focus on D_3.

The metabolic activation of vitamin D_3 is carried out by specific cytochrome P-450 containing enzymes. First, vitamin D_3 passes through the liver and is metabolized to $25[OH]D_3$ (calcidiol) by the action of 25-hydroxylase. Then $25[OH]D_3$ is metabolized to $1,25[OH]_2D_3$ by the action of 1alpha-hydroxylase in the kidney. Both of these enzymes are located in the inner mitochondria in the kidney cells. The synthesis of $1,25[OH]_2D_3$ by the renal 1alpha-hydroxylase appears to be tightly regulated by levels of plasma $1,25[OH]_2D_3$ and calcium. This renal enzyme is induced by the parathyroid hormone (PTH).

Excretion

Both synthesis and degradation of vitamin D are tightly regulated. Catabolism of vitamin D involves 24-hydroxylase which is a third, vitamin D related mitochondrial cytochrome P-450 enzyme and is involved in the catabolism of $25[OH]D_3$ to $24,25\ [OH]_2D_3$. This enzyme also catalyses $1,25[OH]_2D_3$ to $1,24,25[OH]_3D_3$. Both $24,25[OH]_2D_3$ and $1,24,25[OH]_3D_3$ are

ultimately excreted after metabolism. 24-Hydroxylase is strongly induced in target cells by $1,25[OH]_2D_3$ and it prefers 1, 25 $[OH]_2D_3$ to $25[OH]D_3$ as a substrate. This hydroxylation by 24-hydroxylase is now known to occur in all vitamin D target tissues including enterocytes, osteoblasts, keratinocytes and parathyroid cells.

UVB Induced Synthesis of Active Vitamin D ($1,25[OH]_2D_3$) in Skin and Its Significance

Epidermal synthesis of calcitriol under influence of UVB regulates important cellular functions in keratinocytes and immunocompetent cells. The antiproliferative and prodifferentiating effects of calcitriol and other vitamin D analogues are highly effective in the treatment of psoriasis vulgaris.

The known antipsoriatic effects of sunlight could in part be mediated via UV-B induced synthesis of calcitriol. Vitamin D synthesis is also of high importance for the prevention of a broad variety of diseases, including various malignancies.

Also, the discovery of 1 alpha-hydroxylase in the central nervous system (CNS) suggests that the CNS can synthesize the active form of vitamin D (7). Thus, serum 25-hydroxycholecalciferol levels may also influence paracrine production of 1, 25 dihydroxycholecalciferol directly in the CNS (8-10).

Mechanisms of Action of Vitamin D

Vitamin D metabolites are bound in the circulation to vitamin D binding proteins. The active metabolite enters the target cells and binds to vitamin D receptors (VDRs), which are nuclear receptors.

This complex, forms a heterodimer with a retinoid receptor and binds to the vitamin D responsive element on a responsive gene leading to gene expression, either up regulation or down-regulation of gene products such as calcium binding protein or osteocalcin, a process that might take anywhere from hours to days. On the other hand, 1, $25[OH]_2D_3$ may also work through a plasma membrane receptor and a second messenger such as Mitogen-Activated Protein (MAP) Kinase or Cyclic Adenosine Monophosphate (cAMP) and may influence calcium channels (11). The rapid response through a second messenger includes the effects on the pancreas beta cells, on vascular smooth muscle, on the intestines and on monocytes.

Functions of Vitamin D

A key function of $1,25[OH]_2D_3$ is to increase calcium absorption from the intestine. For calcium absorption, longitudinal bone growth, osteoblast and osteoclast activity, both $1,25[OH]_2D_3$ and VDR are essential (12). Genes up-regulated by 1, 25 $[OH]_2D_3$ include osteocalcin, osteopontin, calbindin, 24-hydroxylase and others (13). Metabolites of the active form of vitamin D, down regulate inflammatory markers such as IL-1 and IL-12 and have an antiproliferative effect. They also decrease Parathyroid Hormone (PTH) and Parathyroid Hormone-related Protein (PTHrP) through a negative vitamin D responsive element (13). In summary, the active metabolite $1,25[OH]_2D_3$ stimulates calcium absorption, decreases PTH secretion, stimulates osteoclastic bone resorption, stimulates the osteoblasts, decreases the production of collagen type I, influences muscular function, stimulates cell differentiation and

[Chapter continues on page 74 of: Environment, Mood Disorders and Suicide]

Vitamin D news

Research reveals link between low vitamin D and military suicide

07 January 2013

Research published this past week is the first to report that low vitamin D levels are associated with an increased risk for suicide in US military personnel.

John C. Umhau, MD, and colleagues in Bethesda, Maryland conducted a prospective, case-control study using serum samples stored in the Department of Defense Serum Repository. The researchers matched 495 verified suicide cases to 495 controls by rank, age and sex.

The researchers found that more than 30% of all participants had vitamin D levels below 20 ng/ml. The subjects with the lowest vitamin D status (<15.5 ng/ml) had the highest risk of suicide, while participants with higher 25(OH)D status showed a decreased risk. The authors conclude,

> *"Future studies could determine if additional sunlight exposure and vitamin D supplementation might reduce suicide by increasing 25(OH) D levels."*

Source:

Umhau JC, et al. Low vitamin D status and suicide: A case-control study of active duty military service members. PLOS ONE. Jan 2013.

Page last edited: 07 January 2013

Is low vitamin D linked to military suicide?

Posted on January 10, 2013 by John Cannell, MD

The authors studied 495 cases of suicide among active duty military personnel who had their blood drawn within 2 years of their suicide. They compared them to 495 matched cases controls.

Umhau JC et al. Low Vitamin D Status and Suicide: A Case-Control Study of Active Duty Military Service Members. Plos One

More than 30% of the soldiers had vitamin D levels lower than 20 ng/ml, even in the summer. When sampled in the winter, more than 60% of the soldiers had levels less than 20 ng/ml. They then grouped the soldiers in octiles; in other words, they divided the soldiers into 8 equal groups by grouping them according to vitamin D levels. **They found that soldiers with the lowest levels of vitamin D were twice as likely to complete suicide as were soldiers with higher levels.**

The authors made the following points in their paper:

- Sunlight may exert benefits over and above that of making vitamin D. For instance, sunlight is involved in melatonin physiology and melatonin can affect mood.
- Low vitamin D status has recently been connected with, low cognitive performance, psychotic-like symptoms, and depression.
- A depressive episode does not always precede suicide. The development of suicidal thoughts can be sudden and occur within 10 minutes of a suicide attempt. Impulsivity plays a major role in military suicides.
- Low serotonin occurs during the winter; and as most know, serotonin is popularly thought to be central to feelings of happiness. This fact may confound the relationship between vitamin D levels and risk of suicide.
- **→A recent study found the vitamin D levels of soldiers in basic training in South Carolina fell at the end of 8 weeks of basic training due to the heavy clothing worn by soldiers. ←**

Dr. Umhau and colleagues concluded,

"Studies are urgently needed to develop an appropriate strategy to insure that service members do not suffer the ill effects of a preventable deficiency of vitamin D."

We agree but would add that the military should take immediate steps to treat vitamin D deficiency that is rampant among their soldiers.

BMJ open accessible medical research

Vitamin D_3 supplementation in patients with frequent respiratory tract infections: a randomised and double-blind intervention study

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ABSTRACT

Background: Low serum levels of 25-hydroxyvitamin D_3 are associated with an increased risk of respiratory tract infections (RTIs). Clinical trials with vitamin D_3 against various infections have been carried out but data are so far not conclusive. Thus, there is a need for additional randomised controlled trials of effects of vitamin D_3 on infections.

Objective: To investigate if supplementation with vitamin D_3 could reduce infectious symptoms and antibiotic consumption among patients with antibody deficiency or frequent RTIs.

Design: A double-blind randomised controlled trial.

Setting: Karolinska University Hospital, Huddinge.

Participants: 140 patients with antibody deficiency (selective IgA subclass deficiency, IgG subclass deficiency, common variable immune disorder) and patients with increased susceptibility to RTIs (>4 bacterial RTIs/year) but without immunological diagnosis.

Intervention: Vitamin D_3 (4000 IU) or placebo was given daily for 1 year.

Primary and secondary outcome measures: The primary endpoint was an infectious score based on five parameters: symptoms from respiratory tract, ears and sinuses, malaise and antibiotic consumption. Secondary endpoints were serum levels of 25-hydroxyvitamin D_3, microbiological findings and levels of antimicrobial peptides (LL-37, HNP1–3) in nasal fluid.

Results: The overall infectious score was significantly reduced for patients allocated to the vitamin D group (202 points) compared with the placebo group (249 points; adjusted relative score 0.771, 95% CI 0.604 to 0.985, p=0.04).

Limitations: A single study centre, small sample size and a selected group of patients. The sample size calculation was performed using p=0.02 as the significance level whereas the primary and secondary endpoints were analysed using the conventional p=0.05 as the significance level.

Conclusions: Supplementation with vitamin D_3 may reduce disease burden in patients with frequent RTIs.

ARTICLE SUMMARY

Article focus

- Recent evidence suggests that vitamin D_3 has potent extraskeletal effects, such as suppression of inflammation and strengthening of mucosal immunity by induction of antimicrobial peptides.
- Data from observational studies suggest that low levels of 25-hydroxyvitamin D_3 are associated with an increased risk of respiratory tract infections.
- Results from a limited number of randomised controlled trials on the protective role of vitamin D_3 against respiratory tract infections are inconclusive and thus additional studies are warranted.

Key messages

- Therefore we designed and carried out a randomised controlled trial where a large dose (4000 IU) of vitamin D_3 was given to patients with an increased susceptibility to infections for 1 year.
- The main conclusion is that vitamin D_3 supplementation reduces symptoms and antibiotic consumption among patients with an increased frequency of respiratory tract infections. Thus, vitamin D_3 supplementation may be an alternative strategy to reduce antibiotic use among patients with recurrent respiratory tract infections.

Strengths and limitations of this study

- A high daily dose of vitamin D_3 was used, the study time was a full year covering all seasons and patients with an increased frequency of respiratory tract infections were studied.
- A single study centre, small sample size (n=140) and a selected group of patients.

INTRODUCTION

Vitamin D was discovered when it was noted that rachitic children were improved by exposure to sunlight.[1] It was later shown by Holick *et al*[2] that vitamin D_3 is synthesised in the skin under the influence of ultraviolet light. Vitamin D_3 is further hydroxylated in the liver

to 25-hydroxyvitamin D_3, which is considered to reflect the vitamin D status of an individual patient.[3] The final activation to the active form 1,25-dihydroxyvitamin D_3 ($1,25(OH)D_3$) requires 1-α-hydroxylase activity. This enzyme (also designated CYP27B1) is expressed in the kidney but also in many other cell-types, including epithelial and immune cells.[4] The active vitamin D_3 ($1,25(OH)D_3$) binds to the vitamin D receptor (VDR), which belongs to the nuclear receptor family. Active vitamin D_3 is only present in minute amounts in the circulation and local activation in target cells is crucial for vitamin D-mediated effects on the immune system.[5]

Low levels of 25-hydroxyvitamin D_3 are associated with an increased risk of tuberculosis[6–8] and respiratory tract infections.[9] The mechanism is not fully elucidated but vitamin D_3 has been shown to induce antimicrobial peptides in immune cells.[10] In addition, active vitamin D_3 ($1,25(OH)D_3$) has broad anti-inflammatory effects on the adaptive immune system by shifting the T helper cell pool from a Th1/Th17-response to a Th2/Treg-dominated response.[11 12] Vitamin D_3 has also been shown to suppress the Th2-response in allergic bronchopulmonary aspergillosis.[13] Thus, vitamin D_3 modulates both the adaptive and innate immune system.[14] The bulk of data on vitamin D_3 and infections stems from in vitro experiments and retrospective observational studies. Results from randomised controlled trials (RCTs) where the effects of vitamin D_3 on infections have been investigated (reviewed by Yamshchikov *et al*[15]) are not conclusive and larger clinical trials are therefore warranted.

We designed a study to test the hypothesis that 4000 IU of vitamin D_3 given daily to patients with antibody deficiency and frequent respiratory tract infections for 1 year could prevent or ameliorate infections. In addition, we investigated whether genetic polymorphisms in genes involved in the effect and/or metabolism of vitamin D_3 have an influence on the outcome of vitamin D_3 supplementation.

METHODS

Study design

A prospective, randomised, double-blind placebo-controlled study of vitamin D_3 supplementation in patients with an increased susceptibility to respiratory tract infections. The study was approved by the local Ethical Committee and the Swedish Medical Product Agency and was performed in accordance with the declaration of Helsinki. Written informed consent was obtained from all study participants. The study was registered at www.clinicaltrials.gov prior to inclusion of the first patient (NCT01131858). The EudraCT number is 2009-011758-16. The full protocol is available from the corresponding author upon request.

Sample size calculation

The sample size was based on the assumption that the intervention would reduce the number of days with symptoms from 42 (210 points) to 28 days (140 points), that is, a reduction of the infectious burden by 30%. Given this assumption, a sample size of 60 patients per study group was predicted to provide the study 90% power at a significance level of p=0.02 (Student's t test). To compensate for predicted exclusion of participants, the groups were increased to include 70 patients per treatment arm. Importantly, the significance level of p=0.02 was chosen in the power calculation to ensure that a sufficient number of patients were recruited in order to avoid a type II error in the primary analysis. However, the conventional and widely accepted significance level of p=0.05 was used for statistical analyses of the primary and secondary endpoints.

Participants

Patients at the Immunodeficiency Unit, Karolinska University Hospital, Huddinge, Sweden, were included between March and June 2010 by the study nurses (SH, ML and KJ). Inclusion criteria were age 18–75 years and an increased susceptibility to respiratory tract infections; that is, >42 days with symptoms from the respiratory tract during a 12-month period prior to study inclusion. Patients registered at the Immunodeficiency Unit are closely followed up with a diary of symptoms and antibiotic consumption. Thus, the patients are trained and used to apply such an instrument to assess their infectious status. Data from patients' standard diary were used as an instrument in order to assess patients for eligibility, both via telephone and by the responsible physician (PB and ACN) prior to inclusion. Patients with selective IgA-deficiency (*D80.2*), IgG-subclass deficiency (*D80.3*) and common variable immune disorder (CVID, *D83.0*) as well as patients without a defined immunological diagnosis (*D89.9*) were included. Exclusion criteria were prophylactic treatment with antibiotics, history of hypercalcaemia or stones in the urinary tract, sarcoidosis, ongoing supplementation with vitamin D_3 exceeding 400 IU/day, HIV-infection and pregnancy.

Interventions

Patients were randomised to 12 months' treatment with vitamin D_3 (Vigantol, 4000 IU/day, Merck GmbH, Darmstadt, Germany) or placebo oil. One drop contained 500 IU vitamin D_3 or placebo oil (Miglyol oil, Merck GmbH, Darmstadt, Germany) and the participants were asked to take eight drops daily. The participants had to mark their daily symptoms of infection in a diary, which was sent via regular mail to the study site every month. The following data were recorded: symptoms from the respiratory tract, ears and sinuses, treatment with antibiotics, numbers of bacterial cultures, times and reasons of visits to hospitals, frequency of travelling abroad and adherence to study drug.

Outcomes

The primary outcome was a composite infectious score, based on a daily patient-reported questionnaire and

included five parameters: symptoms from the respiratory tract, ears and sinuses, malaise and use of antibiotics (see online supplementary figure S1), each parameter gave 1 point/day. The occurrence of x-ray verified pneumonia gave three additional points per day for a period of 7 days. Thus, a pneumonia resulted in 3×7 points=21 extra points. Patients were specifically instructed to record only symptoms related to ongoing respiratory tract infections. Symptoms related to infections at other sites (urinary tract, wounds, etc) as well as non-infectious symptoms were reported as adverse events. Secondary outcomes were serum levels of 25-hydroxyvitamin D_3 (at baseline and after 3, 6, 9 and 12 months), numbers of bacterial cultures, microbiological findings and levels of antimicrobial peptides (LL-37 and HNP1-3) in nasal fluid (at baseline and after 6 and 12 months). In addition, six post hoc genotype analyses were performed in all participants. Analyses of single nucleotide polymorphisms (SNPs) were carried out for VDR (TaqI and FoqI), CYP27B1, CYP24A1, CYP2R1 and vitamin D binding protein (GC). Safety tests included plasma levels of creatine, calcium, phosphate and albumin, measured at baseline and after 3, 6, 9 and 12 months. At inclusion, urine-HCG (human chorionic gonadotropin) in women was measured and p-parathyroid hormone was measured in both genders. The results of the safety tests were reviewed by an independent and unblinded consultant physician. Two blinded physicians (PB and ACN) were responsible for inclusion and all medical visits to the study site (Immunodeficiency Unit, Karolinska University Hospital, Huddinge, Sweden).

Randomisation and statistical analysis

Participants were randomised to 12 months' treatment with vitamin D_3 (Vigantol, 4000 IU/day) or placebo oil. Block randomisation with a block size of ten was used to ascertain equal group sizes. Staff at Karolinska Trial Alliance was responsible for randomisation procedures. In the statistical analysis, continuous variables were compared using Mann-Whitney U test or linear regression and dichotomous variables by Fisher's exact test or logistic regression. Regressions of log-transformed infectious scores were performed both unadjusted (simple regression) and with adjustment for potential confounders (multiple regression).

Statistical methods: primary analysis

The distribution of the infectious score was found to be skewed, thereby violating the normal assumption of the prespecified t test analysis. Hence, scores were log-transformed prior to analysis. Further, the randomisation had resulted in age distributions that were not entirely balanced between the two groups. Since there might be concerns that such imbalance could influence the results of the study, the original analysis plan was extended with a multivariable analysis adjusting for potential confounders. In this linear regression model based on log-transformed values of the primary outcome (the total infectious score) and its individual components, adjustment was made for age, gender, smoking, type of immune deficiency and significant comorbidities (respiratory or non-respiratory). Because of the transformation procedure, the adjusted effect of vitamin D_3 is expressed as a ratio between the score in the vitamin D_3 and the placebo group. In this multiplicative model, an effect size of 1 indicates identical outcome in the two study groups and statistically non-significant results are recognised by CIs encompassing the value 1.

To explore potential divergent effects on different organ systems, both adjusted and unadjusted analyses were repeated separately for each individual item of the infectious score. In addition, the temporal aspects of the vitamin D_3 effect were investigated by dividing the study period into four 90-day periods (starting on the first day of treatment) and repeating the analyses separately for each time period. 'Ear' and 'sinus' symptoms as well as 'antibiotic use' occurred at low frequencies and for these entities normal distributions could not be achieved despite data transformation. Thus, the adjusted analyses of these individual items were based on multivariable logistic regression, after coding the symptom (or antibiotic therapy) as present or absent during the course of the study. However, this only applies to analysis of the individual items, and not to the primary analysis of the total infectious score, where all item scores were added as originally described.

Most postrandomisation exclusions were due to patients failing to fill out the symptoms diary. Hence, no intention-to-treat (ITT) analysis based on actual outcome data could be performed. However, the potential impact of dropouts was addressed in an ITT analysis based on multiple imputation of missing outcome data. In the imputation process, pooled estimates were derived from 100 datasets created by means of multivariate imputation by chained equations and predictive mean matching for the same covariates as in the adjusted per-protocol analysis.

Detailed descriptions of randomisation and blinding, sampling of nasal fluid, measurement of antimicrobial peptides, measurement of 25-hydroxyvitamin D_3, genotyping and statistical analyses of secondary outcomes are presented in the supplementary methods section.

RESULTS

Baseline data

A total of 286 patients were first assessed for eligibility but 144 were not included because they did not fulfil all inclusion criteria; <42 days with infection/year (n=35), lacked other inclusion criteria (n=42), or declined to participate (n=67). The remaining 142 patients were further screened and 140 patients were included in the study. Of these, 70 were randomised to vitamin D_3 supplementation and 70 to placebo (figure 1). The groups did not differ with regard to gender, IgG replacement therapy, smoking, baseline 25-hydroxyvitamin D_3 levels,

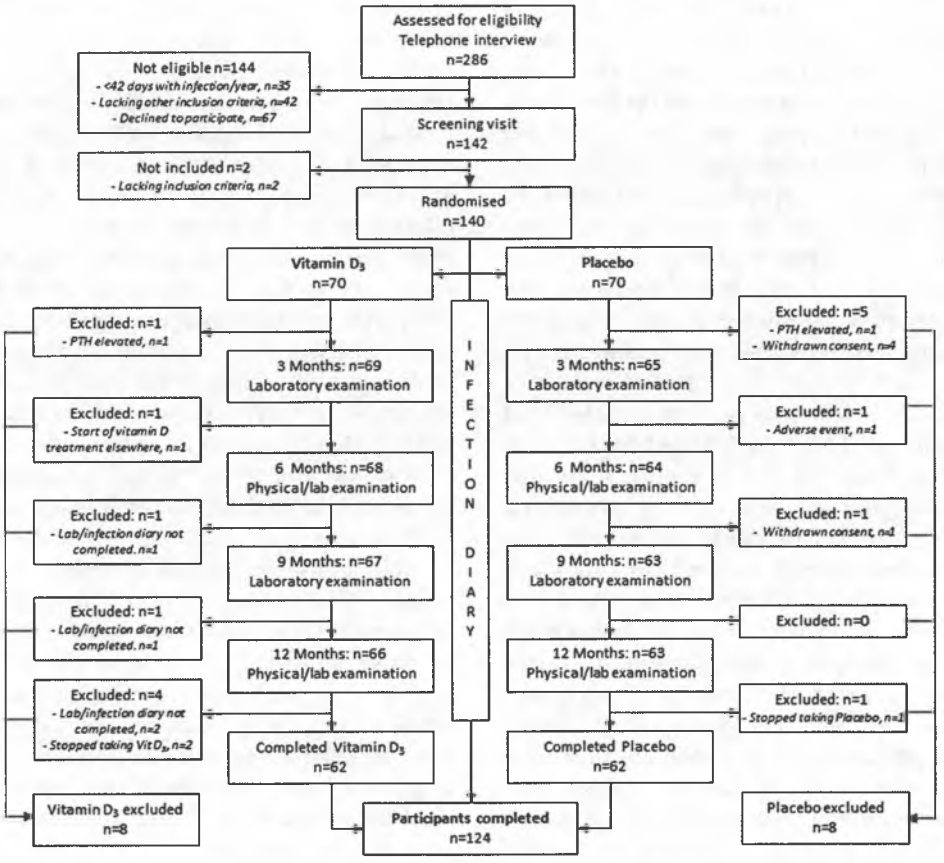

Figure 1 Study outline.

type of immune defect or comorbidities (table 1). Patients with subclass deficiency, selective IgA deficiency (sIgAD), CVID and patients without a defined immunological diagnosis (ND) but with >4 bacterial respiratory tract infections/year were included. IgG replacement therapy was most common in the CVID group (100%) and in the subclass deficiency group (63%), and also frequent in the other groups (ND, 54% and sIgAD, 38%, see online supplementary table S1). Patients allocated to the placebo group were slightly younger than patients in the treatment group (p=0.025, data not shown). During the course of the study, 16 patients left the study prematurely (8 patients from each study group) and consequently 124 patients were included in the main per-protocol analysis. Reasons for dropout included elevated parathyroid hormone (n=2), withdrawn consent (n=5), adverse events (n=1), prescription of vitamin D outside the study (n=1), failure to complete diary (n=4) or non-compliance to study medication (n=3; figure 1).

Primary endpoint: infectious score

One year of vitamin D_3 treatment was associated with a significantly reduced total infectious score both in the unadjusted (n=124, p=0.024; table 2) and in the adjusted analyses (n=124, p=0.040; table 2; figure 2A,B and see online supplementary table S2). The

Table 1 Baseline data

	Vitamin D_3	Placebo
Number	70	70
Age (mean)	55.4	50.8
Female	52/70	50/70
Male	18/70	20/70
IgG-replacement	39/70	42/70
Smoking	4/70	6/70
25-OH levels (mean) (nmol/l)	51.5	46.9
Immunological diagnosis		
sIgA-deficiency	9/70	9/70
IgG subclass	27/70	30/70
CVID	6/70	4/70
ND	28/70	27/70
Concomitant disease		
No other disease	16/70	18/70
Lung: Asthma	27/70	25/70
Lung: BE	5/70	7/70
Lung: COPD	5/70	4/70
Other disease*	17/70	16/70

Mann-Whitney U test was used for comparisons of age and 25-OH vitamin D_3. Fisher's exact test was used for all other comparisons.
*'other disease' includes hypertension, body pain, hypothyroidism and gastritis as most common diagnoses.
BE, bronchiectasis; COPD, chronic obstructive pulmonary disease; CVID, common variable immuno deficiency; ND, increased susceptibility to infections without a defined immunological disorder.

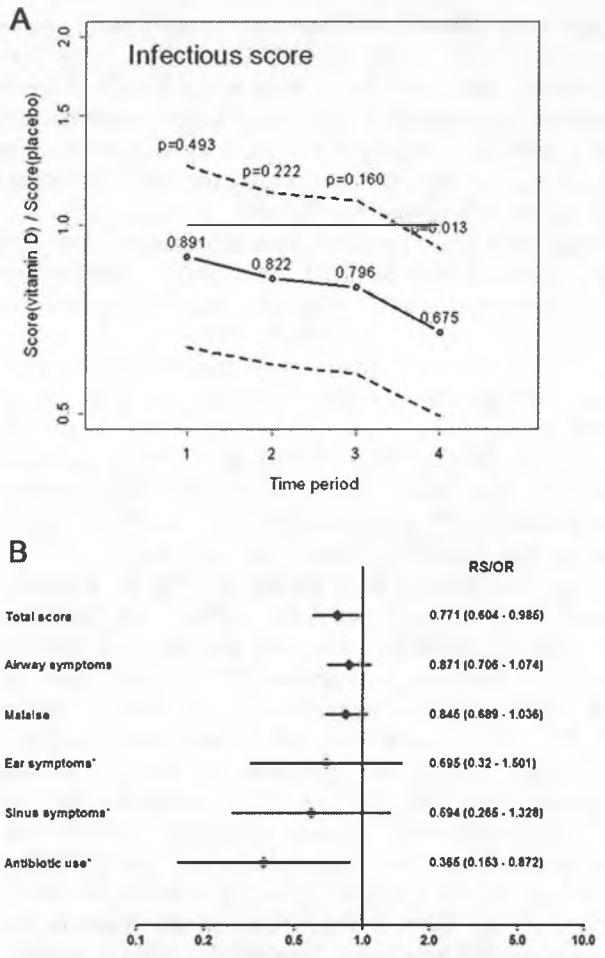

Figure 2 Primary endpoint. The adjusted total relative infectious score (A) is expressed 'per quarter' (3-month periods). The adjusted 1-year scores (total score, airway, malaise, ear, sinus and antibiotics) are depicted in a Forest-plot (B) together with 95% CI. Effects are presented as relative scores (total score, airway and malaise) or OR (ear, sinus, antibiotics and indicated with asterisks).

unadjusted relative score in the intervention group was 0.754 (95% CI 0.591 to 0.963, p=0.024, n=124) corresponding to a 25% reduction and after adjustment for potential confounders, the relative score was 0.771 (95% CI 0.604 to 0.985, p=0.04), corresponding to a 23% reduction (table 2). According to the temporal analysis, the effect of vitamin D_3 supplementation tended to improve with time (figure 2A). The absolute unadjusted score per patient was 202 points for the vitamin D group and 249 points for the placebo group, a significant reduction of 47 points per patient (p=0.023, Mann-Whitney U test, see online supplementary table S3).

When the individual items of the infectious score were analysed separately, all point estimates indicated a reduction in the treatment group (table 2, see online supplementary figure S2), although only antibiotic consumption reached statistical significance (figure 2B and see online supplementary figure S2, panel E). The adjusted OR for antibiotic use was 0.365 (95% CI 0.153 to 0.872, p=0.023, n=124), that is, a 63.5% reduction of the odds of antibiotic use in the intervention group (table 2). The absolute values were 33 days on antibiotics for the placebo group and 16 days for the vitamin D_3 group, that is, a reduction of 17 days in the vitamin D_3 group (see online supplementary table S3). The temporal trends for specific symptoms and antibiotic consumption were similar to the total score and reached statistical significance for 'ear'-symptoms (n=124, p=0.041) and for 'malaise' (n=124, p=0.053) in the final quarter of the study (see online supplementary figure S2, panels B and C).

Analysing the primary outcome according to ITT (n=140) produced results virtually identical to those of the per-protocol analysis. In the unadjusted ITT analysis, vitamin D_3 reduced the total infectious score by 25% (relative score 0.752, 95% CI 0.588 to 0.962, p=0.024) and after adjustment for potential confounders the reduction was 23% (relative score 0.767, 95% CI 0.599 to 0.982, p=0.036).

Serum levels of 25-hydroxyvitamin D_3

Serum 25-hydroxyvitamin D_3 levels did not differ between the groups at baseline (table 1) but already after 3 months

Table 2 Primary endpoint

	Univariable regression model (unadjusted values)			Multiple regression model (adjusted values)		
Endpoint	**Effect**	**95% CI**	**p Value**	**Effect**	**95% CI**	**p Value**
Total score	0.754	0.591 to 0.963	0.024	0.771	0.604 to 0.985	0.040
Airway	0.857	0.697 to 1.053	0.141	0.871	0.706 to 1.074	0.200
Ear*	0.721	0.352 to 1.465	0.367	0.695	0.320 to 1.501	0.357
Sinus*	0.583	0.280 to 1.198	0.144	0.594	0.265 to 1.328	0.204
Malaise	0.845	0.692 to 1.032	0.098	0.845	0.689 to 1.036	0.108
Antibiotics*	0.355	0.154 to 0.784	0.012	0.365	0.153 to 0.872	0.023

Treatment effect calculated as the ratio between infectious scores in the vitamin D_3 and the placebo groups. Due to low frequencies, endpoints marked with asterisks (*) were coded as binary outcomes (ie, present or absent in each patient) and compared by means of logistic regression. In these cases, the effect refers to OR of experiencing the outcome at least once during the course of the study (The data are based on n=124 patients).

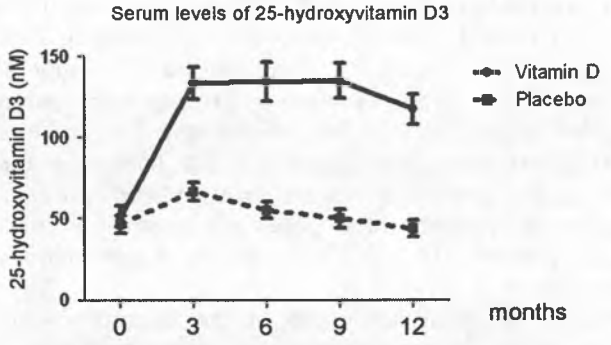

Figure 3 Secondary endpoint. Vitamin D levels. Serum was collected at days 0, 3, 6, 9 and 12 months and levels of 25-hydroxyvitamin D_3 were measured. Values are expressed as mean±95% CI.

the intervention group had a significantly higher level of 25-hydroxyvitamin D_3 (133.4 vs 66.6 nmol/l, p<0.001; figure 3). This increase remained throughout the study (figure 3).

Bacterial cultures and microbiology

During the course of the study, 173 microbiological samples were obtained in the vitamin D_3 group (n=62, 2.79/patient) and 301 in the placebo group (n=62, 4.85/patient; p=0.010; table 3). The number of samples with at least one positive finding was higher in the placebo group, with close to statistical significance (p=0.052), while the fraction of positive samples was similar for both groups (table 3). Significantly more patients had a microbiological sample taken from the respiratory tract (≥1 sample) during the study period in the placebo group; OR 2.63 (95% CI 1.17 to 5.92; table 3).

In total, the vitamin D_3 group generated 76 positive microbiological findings (bacteria or fungi), compared with 159 in the placebo group (p=0.023). There was no difference between the groups for the traditional respiratory pathogens (*Haemophilus influenza, Moraxella catharralis* and *Streptococcus pneumonia*), but there were significantly fewer findings of *Streptococcus aureus* (p=0.019) and fungi (p=0.028, *Candida* spp. and *Aspergillus* spp.) in the treatment group (table 4). Likewise, significantly fewer vitamin D_3-treated patients had a bacterial culture positive for *S aureus* (p=0.019) or fungal species (p=0.058), although the latter difference did not reach statistical significance (table 4).

Vitamin D treated patients with subclass deficiency left significantly fewer bacterial or fungal cultures than placebo-treated patients with this diagnosis; seven cultures in the vitamin D group (n=22) versus 47 cultures in the placebo group (n=24) (see online supplementary table S4). Also the number of patients that had ≥1 bacterial culture taken was significantly fewer in the placebo group (12/22 vs 22/24, p=0.0065, see online supplementary table S4). There was no significant effect of other immunological diagnoses on bacterial cultures or microbiology (see online supplementary table S4).

Since concomitant lung disease may be an important factor for vitamin D mediated effects on respiratory immunity, we performed a detailed analysis of bacterial cultures and microbiology of patients with asthma, bronchiectasis (BE) and chronic obstructive pulmonary disease (COPD). The numbers of patients with these diagnoses were quite small, which preclude any firm conclusions regarding any effect. However, there was a trend—however not significant—that vitamin D-treated patients with asthma produced fewer bacterial cultures (average 2.9 cultures/patient vs 7.0 cultures/patients, p=0.080, see online supplementary figure S3) and fewer positive cultures than placebo-treated asthmatics (average 0.6 positive cultures/patients vs 2.7/patient in the placebo group, p=0.052, see online supplementary figure S3). In addition, vitamin D-treated asthma patients showed significantly fewer cultures positive for fungi (*Candida* and *Aspergillus*) compared with placebo-treated asthmatics (p=0.0476, see online supplementary table S5). For BE or COPD patients there was no clear trend or significant effect in bacterial cultures or microbiology.

Table 3 Bacterial cultures

	Vitamin D_3	Placebo	Significance
Number of samples per patient (mean, n=62/62)	2.79	4.85	p=0.010*
Number of positive samples per patient (mean, n=62/62)	1.01	2.02	p=0.052*
Fraction positive cultures (%)	63/173 (36%)	125/301 (41%)	p=0.28**
Patients with ≥1 sample taken	38/62 (61%)	50/62 (81%)	p=0.029**

*Mann-Whitney U test.
**Fisher's exact test.

Levels of antimicrobial peptides in nasal fluid

There was no statistically significant difference between the vitamin D_3 or placebo groups when nasal fluids were analysed for the presence of antimicrobial peptides (AMPs). Initially, the levels of both LL-37 and HNP1-3 tended to be higher in the placebo group (see online supplementary figure S3, panels A and B). However, after 12 months the microbiological pattern was reversed and no primary pathogens could be detected in nasal swabs from vitamin D_3-treated patients (n=25, p=0.039; see online supplementary figure S4, panel C). The placebo-treated patients exhibited the same mix between normal flora and primary pathogens at all three sampling points (0, 6 and 12 months; see online supplementary figure S4, panel C).

SNP variants and treatment effect

Most genetic variants did not affect the primary endpoint. However, patients carrying the 'AA' genotype in

Table 4 Microbiological findings

Microorganism	Number of findings (total)			Number of patients		
	Vitamin D_3	Placebo	MW-U	Vitamin D_3	Placebo	Fisher
Haemophilus influenzae	28	27	p=0.46	10/62	13/62	p=0.64
Moraxella catharralis	8	17	p=0.39	7/62	10/62	p=0.60
Streptococcus pneumoniae	7	6	p=0.74	4/62	5/62	p=1.00
Staphylococcus aureus	6	33	p=0.010	4/62	14/62	p=0.019
Enterobacteriacae	8	8	p=0.39	4/62	7/62	p=0.53
Pseudomonas aeruginosa	8	15	p=0.68	3/62	4/62	p=1.00
Fungal infection	11	53	p=0.028	4/62	12/62	p=0.058
Total	76	159	p=0.023			

Mann-Whitney U test was used to analyse the total number of findings, whereas Fisher's exact test was used for analysis of the number of patients (fraction) with a specific finding.

the CYP2R1-gene, encoding the 25-hydroxylase enzyme, had a larger benefit of vitamin D_3-supplementation (–55%) compared to AG or GG carriers (–6%) (n=124, p=0.046 for interaction, see online supplementary table S6).

Adverse events

In total, the vitamin D_3 group reported 38 adverse events (AEs) versus 56 AEs in the placebo group. The most common symptoms in the treatment group were headache (n=5) and lumbago (n=5), whereas placebo-treated patients reported paresthesias (n=8), diverticulitis (n=4) and urinary tract infection (n=4) as most frequent AEs (table 5, see online supplementary table S7). There was a general trend towards the number of adverse events being higher in the placebo group. Significantly more patients in the placebo group reported cardiovascular problems, such as heart failure, hypertonia and thrombosis (p=0.028). For gastrointestinal and other (non-respiratory) infections there was also a trend favouring the vitamin D_3 group (p=0.058 and 0.09, respectively). No clinically relevant changes in serum levels of calcium, phosphate, creatine or albumin could be observed (see online supplementary figure S5). There was one severe adverse event in each group (rabdomyosarcoma in the vitamin D_3 group and lung bleeding in the placebo group), both judged as being unrelated to the study drug.

Table 5 Adverse events

Organ	Vitamin D_3 n (%)	Placebo n (%)	p Value
CNS	11 (29)	10 (18)	1.00
Gastrointestinal	4 (11)	12 (21)	0.058
Cardiovascular	0 (0)	6 (11)	0.028
Infections (other than RTI)	2 (5)	8 (14)	0.09
Musculoskeletal	10 (26)	10 (18)	1.00
Respiratory (non-infectious)	2 (5)	4 (7)	0.68
Skin	5 (13)	2 (4)	0.44
Other	4 (10)	4 (7)	1.00
Total	38	56	

Number of reports. Fisher's exact test was used for between group comparison (the data are based on AE-reports from n=62 patients/arm).
CNS, central nervous system, RTI, respiratory tract infection.

DISCUSSION

The main conclusion from this long-term RCT is that vitamin D_3 supplementation reduces the total burden of respiratory tract infections. The primary endpoint was composed of five different parameters that patients recorded daily throughout the study year. All point estimates favoured the vitamin D_3 group and a statistically significant effect was seen on both the total score and on the probability of receiving antibiotics (p<0.05). The effect on the infectious score was evident both in analysis per-protocol and according to ITT, and withstood adjustment for potential confounders. In addition, the number of bacterial cultures and microbiological findings was significantly reduced in the intervention group. These findings are potentially important and support that Vitamin D_3 supplementation may prevent respiratory tract infections and reduce antibiotic consumption, particularly in patients with hypogammaglobulinaemia or with an increased frequency of respiratory tract infections.

However, our study has several limitations: First, the choice of primary endpoint may be questioned since it relies solely on patient-reported information. To compensate for inherent problems with patient-reported data, the evaluation instrument was designed to cover many aspects of an infectious episode, including various symptoms as well as antibiotic consumption. Together the reported data formed an 'infectious score', which constituted the primary endpoint of the study. Similar composite scores have successfully been applied to different diseases, such as tuberculosis (TB-score[16]), pneumonia (CURB-65[17]) and bacterial meningitis (BMS-score[18]). Notably, vitamin D supplementation had a major effect on the odds of taking antibiotics during the study period (a reduction by 63.5%). In addition, the absolute number of days on antibiotics was reduced by 50% (from 33 days in the placebo group to 16 days in

the intervention group), which was statistically significant both in the adjusted and unadjusted analyses (table 1). However, despite the relatively modest reduction for the other components of the primary endpoint the overall infectious score was significantly reduced—mainly as a result of the large effect on the antibiotic parameter—both in the unadjusted and in the adjusted analyses (table 1 and figure 2). It is important to interpret the statistical significance in light of our power calculation, which was based on a significance level of p=0.02. In the power calculation, the significance level was reduced from 0.05 to 0.02 in order to increase the statistical power at the p=0.05 level. This approach was incorrect, and the targeted power (at the p=0.05 level) should instead have been increased without altering the p-value threshold. However, we have used the widely accepted significance level p=0.05 in the statistical analyses for both the primary and secondary endpoints, respectively. Another potential problem was that the patient population was very heterogeneous with regard to immune deficiency and concomitant diseases. We adjusted for these factors in the multivariable analyses of the primary endpoint, but the sample sizes in each subgroup were too small to draw any conclusions of effects in specific disease groups. However, a detailed post hoc analysis of the relation between immunological diagnosis, concomitant lung disease and the secondary endpoints 'taken bacterial cultures', 'positive bacterial' cultures and 'microbiological findings' was performed. There was a clear trend that vitamin D-treated patients with subclass deficiency and/or asthma produced fewer bacterial cultures, fewer positive cultures and fewer fungal cultures (see online supplementary tables S4 and S5 and figure S3). Although this analysis may lack precision by the small number of patients included, it could have clinical implications regarding target groups for vitamin D_3 supplementation.

Nevertheless, our double-blind RCT has several strengths. For example, we chose a high daily dose of vitamin D_3 based on published calculations on metabolism and effects on immunity.[14 19] Other RCTs using lower doses of vitamin D_3, 400–2000 IU/day, have mainly been negative with regard to the prevention of infections.[20 21] However, one study using 1200 IU/day showed a significant reduction of influenza among school children in Japan.[22] Notably, also studies using higher doses of vitamin D_3 have been negative. Martineau *et al* used 400 000 IU vitamin D_3 during 42 days (9523 IU/day) with the aim of shortening time to sputum conversion in tuberculosis. No significant effect on the primary endpoint could be observed in that study, except in a subgroup with the *tt* genotype in the VDR gene.[23] A recent study investigated whether 100 000 IU vitamin D_3/month (3333 IU/day) could reduce the incidence of COPD exacerbations. There was no significant effect on the primary endpoint, although a post hoc analysis revealed that patients with a low vitamin D_3 level at baseline had a significant effect of vitamin D_3 supplementation.[24]

Importantly, our study is the first to utilise high daily doses for an extended period of one full year. Thus, we covered all four seasons, which was important in Sweden with a known seasonal variation in 25-hydroxyvitamin D_3 levels.[25] Two previous RCTs were performed during the winter season—when vitamin D levels are low—but only during 4[22] and 6 months,[20] respectively. Previous RCTs have been conducted during shorter periods; 42 days,[23] 6 weeks[26] and 12 weeks,[21] respectively. Interestingly, we observed a clear time-dependent effect suggesting that a long-term supplementation approach (>6 months) may be necessary to affect immunity. To expand on the results of a previous study in healthy individuals where no difference between the intervention and placebo groups was observed,[21] we chose a study population with frequent RTIs and at least 42 days with infection during the year prior to inclusion. Notably, patients in the study represent a selected group of individuals with frequent RTI, although the immune disorders that they represent (sIgAD, IgG-subclass deficiency and patients with no defined immune disorder) are generally mild in character and dominated by mucosal RTIs. We also included a small number of CVID-patients, which can be considered to be a more severe immune disorder, but all these patients are treated with IgG replacement therapy and thus well controlled. Hence, the results from this study cannot directly be applied to the general healthy population. Nevertheless, the results provide solid support for additional interventional studies of vitamin D_3, especially in groups consuming large amounts of antibiotics.

The mechanism of the observed effects remains largely unknown. Vitamin D_3 modulates the immune response at many levels, such as induction of AMPs, skewing of T-cells from Th1/Th17 to Tregs as well as general anti-inflammatory effects.[14] Here, we investigated the role of AMPs in nasal fluid. However, we could not detect any significant changes of LL-37 or HNP1-3 during the study period, but noted that placebo-treated patients tended to have higher levels of AMPs after 1 year of treatment. This was paralleled by a shift of the microflora in the nasal compartment that could explain the unexpected finding of higher AMP-levels in the placebo group. Recently, it was shown that 1,25 $(OH)_2$-vitamin D_3 induces both HNP1-3 and LL-37 in nasal fluid of healthy volunteers,[27] supporting that LL-37 may indeed be induced in vivo. However, our study design did not allow such conclusions but rather support that vitamin D_3 affect mucosal immunity, leading to a shift of the microflora. Recently, we showed that the bacterial composition in nasal swabs is an important determinant of AMP-levels in nasal fluid.[28]

Given that vitamin D_3 induces LL-37 in epithelial cells and that LL-37 kills bacteria in vitro, we expected a reduction of the classical bacterial pathogens *H influenza*, *M catharralis* and *S pneumonia* in the intervention group. However, the frequency of these bacteria was not reduced

but a reduction of *S aureus* and fungal species that often colonise the airways was observed. This could be explained by specific effects by vitamin D_3 on immunity against *S aureus*. In fact, vitamin D_3 induces human β-defensin-2 (HBD-2) with bactericidal activity against *S aureus*.[29] A recent study showed that low vitamin D_3 levels were associated with an increased risk of being colonised by this bacterium.[30] Further, vitamin D_3 affects immunity against *C albicans*, which indicates direct effects of vitamin D_3 on human immunity.[31] Alternatively, it is possible that vitamin D_3 may have prevented symptomatic viral infections, which prompted patients to leave a bacterial sample from the airways. Interestingly, there is both mechanistic and clinical evidence that vitamin D_3 can prevent viral infections,[32–34] although we did not address this in the current study.

Notably, we observed a prominent increase in the serum concentration of 25-hydroxyvitamin D_3, which indicated good compliance and tolerability of the study drug. In fact, there was a trend towards adverse events being reported more often in the placebo group, suggesting that vitamin D_3 possibly could be efficient against other diseases, but this observation requires further studies. No clinically relevant changes of blood chemistry (calcium, phosphate, albumin or creatine) were observed. Despite few adverse events and high tolerability, 16 exclusions occurred during the study year. The main reason was problems to adhere to the protocol and 6/16 patients dropped out of the study after a few weeks. The rest failed to send in the diaries, did not leave blood for monitoring of safety parameters or did not take the study drug. One patient was excluded based on symptoms that could be attributed to vitamin D_3 (facial paraesthesia). However, this patient was later confirmed to have been allocated to placebo.

In summary, we found that supplementation with vitamin D_3 reduced the total infectious score with 47 points per patient (23% reduction in the adjusted analysis) during the study year. The observed reduction was lower than the assumed reduction of 70 points per patient (predefined assumption: 210 points=>140 points; a reduction of 30%) that formed the basis for the power calculation. However, despite the predefined level of a reduction of infectious score by 30% as a clinically meaningful effect, we believe that effects lower than this also could be relevant for the individual patient. We base this line of reasoning on the fact that a reduction of 47 points per patient can be translated into 47 days with cough (47 points), 23 days with ear and sinus symptoms (23×2=46 points) or 9 days with cough, sinus and ear symptoms together with malaise and antibiotics (9×5=45 points). In addition, our data indicate that vitamin D_3 supplementation reduces the odds of taking antibiotics by approximately 60% in patients with frequent respiratory tract infections. Thus, supplementation with vitamin D_3 could provide a novel strategy to reduce antibiotic use among high consumers and indirectly prevent the emerging epidemic of bacterial resistance.

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Contributors PB designed the study, collected, analysed and interpreted data, wrote the paper. A-CN designed the study, collected and interpreted data, wrote the paper. SH designed and coordinated the study, collected and interpreted data. RSR carried out experimental work and analysed data. BA analysed and interpreted data, wrote the paper. LB-B analysed and interpreted data, wrote the paper. LE analysed and interpreted data. JL analysed and interpreted data, wrote the paper. JA designed the study, interpreted data, wrote the paper.

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Competing interests None.

Patient consent Obtained.

Ethics approval The study was approved by the local Ethical Committee and the Swedish Medical Product Agency and was performed in accordance with the declaration of Helsinki. Written informed consent was obtained from all study participants.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement There are no additional data available.

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Vitamin D_3 supplementation in patients with frequent respiratory tract infections: a randomised and double-blind intervention study

Peter Bergman, Anna-Carin Norlin, Susanne Hansen, et al.

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Notes

HOUSE BILL NO. 90

IN THE LEGISLATURE OF THE STATE OF ALASKA

TWENTY-EIGHTH LEGISLATURE - FIRST SESSION

BY REPRESENTATIVE SEATON

Introduced: 1/30/13
Referred: Health and Social Services, Finance

A BILL

FOR AN ACT ENTITLED

"An Act establishing a temporary program in the Department of Health and Social Services for testing newborns for baseline vitamin D levels."

BE IT ENACTED BY THE LEGISLATURE OF THE STATE OF ALASKA:

*** Section 1.** The uncodified law of the State of Alaska is amended by adding a new section to read:

NEWBORN TESTING PROGRAM FOR VITAMIN D; LEGISLATIVE FINDINGS. (a) The legislature finds that multiple studies demonstrate a link between vitamin D insufficiency in newborns and higher incidences of mental and physical health problems that lead to higher costs of future medical, educational, and support services.

(b) Beginning on or before January 1, 2014, the Department of Health and Social Services shall establish a 12-month statewide program for testing the vitamin D levels of newborns at birth or as soon after birth as possible for the purpose of acquiring baseline vitamin D levels of all newborns in the state during the testing period. The testing shall be conducted, at no cost to a parent or guardian of the newborn, by or under the supervision of a

health care professional licensed in the state who attends the delivery.

(c) The program established under this section must include a procedure for

(1) combining, to the extent feasible, the vitamin D testing with other newborn or cord blood testing;

(2) ensuring testing complies with federal and state privacy laws;

(3) reporting test results to the department and to the parent or guardian of the newborn; and

(4) permitting a mother of a newborn to refuse testing if seriologic testing is contrary to the tenets or practice of the religious creed of the mother.

(d) The department shall contract with a laboratory that is affiliated with an accredited university in the United States and that is conducting national clinical research on the subject of newborn and prenatal vitamin D levels to provide laboratory and analytic services for the samples collected under this section.

(e) In this section, "health care professional" means a physician, physician assistant, midwife, or nurse.

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REPRESENTATIVE Paul Seaton

District 30

HB 90- Vitamin D Supplements
Sponsor Statement

Recent pediatric studies relating to vitamin D levels and infant development have added to growing evidence of the importance of vitamin D sufficiency in areas such as language, psychomotor, and mental development. These studies demonstrate an association between low vitamin D levels in newborns and higher risks of mental and physical health problems. The studies also connect vitamin D deficiency to several factors relevant to Alaska. Northern latitude, skin pigmentation, and Alaskan's predominantly long sleeve clothing may all lead to low levels in our residents. **HB 90** is a temporary law, establishing a year-long project to test Alaska's newborn vitamin D levels. The samples collected during the HB 90 testing period will be sent to an accredited laboratory to provide testing and analytical services. By testing across the wide spectrum of locations and population groups, we will gain insight into how our population is affected by Alaska's northern latitude and which subgroups are at a greater risk of vitamin D deficiency. Understanding and targeting those at-risk Alaskans revealed by the study at the newborn and neonatal level, when important brain development is occurring, could lead to future savings in medical, educational, and support service costs. Depending on the Alaskan Newborn results, demonstrated links between neural development and vitamin D levels may point to vitamin D deficiency as a limiting factor in reaching our State's educational goals.

Nenana Student Living Center's Response to HJR 5:
Vitamin D supplement program & student absences

Dear Taneeka,

I have attached a sheet with the Vitamin D data from the Nenana Student Living Center. We compared students who live at the Nenana Student Living Center that take Vitamin D to students who live there and don't have parental permission to take Vitamin D. This is the first year we have done this. Students who take Vitamin D miss less days due to illness.

Of the 18 students who have parental permission to be given Vitamin D every day* this year, we also compared the absences due to illness this year with their absences due to illness last year, when we did not offer Vitamin D to our kids. There were 8 students who take Vitamin D this year that were also at the Nenana Student Living Center last year when Vitamin D was not available to kids. Absences due to illness has improved for these kids as well.

If you have any questions about this data or if you can think of any other kinds of comparisons that might help, please let me know. Personally, I take Vitamin D every day and it sure seems like it helps me.

Please let Representative Seaton know that I will help him in any way I can.

Thanks,
Eric Gebhart, Superintendent
Nenana City School District
PO Box 10
Nenana, AK 99760

*We give 1000 IU of Vitamin D, once a day.

NSLC – Vitamin D Data

Total NSLC student count: 68
Vitamin D participants: 18
Non-participants: 50

Participating Statistics

Absent days due to illness:

0 days - 40%
1 day - 11%
2 days – 5%
3 days – 11%
4 days – 17%
5 days – 11%
6 days – 5% (most days missed)

Total days absent by group:
38 days / 1572 total
2.4 %

Non-Participating Statistics

Absent days due to illness:

0 days – 26%
1 day – 14%
2 days – 16%
3 days – 8%
4 days – 14%
5 days – 8%
6 days – 2%
8 days – 4%
9 days – 2%
10 days – 4%
12 days – 2% (most days missed)

Total days absent by group:
145 days / 4517 total
3.2%

This data is based on the first 100 days of school for 2012-13. Transfer student data was based on actual days in membership. This data was generated specifically for days absent due to illness, and does not include absences for doctor's appointments, or other appointments.

Comparison Data for Returning Student Participants

Number of Returning Students: 8

2011-12 Absent days due to illness:

0 days – 25%
2 days – 12.5%
3 days – 25%
4 days – 12.5%
7 days – 12.5%
13 days – 12.5% (most days missed)

Total days absent by group:
32 days / 1288 total
2.5%

2012-13 Absent days due to illness:

0 days – 62.5%
2 days – 12.5%
3 days – 12.5%
4 days – 12.5% (most days missed)

Total days absent by group:
9 days / 800 total
1.1%

This data is based on 170 school days for 2011-12, and the first 100 days of 2012-13.

Vitamin D: Implications for Alaskan Children

Presentation to the Alaska Native Health Board
February 4th, 2013

Representative Paul Seaton

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Australia

Language Impairment

Maternal Serum Vitamin D Levels During Pregnancy and Offspring Neurocognitive Development

Andrew J. O. Whitehouse, Barbara J. Holt, Michael Serralha, Patrick G. Holt, Merci M. H. Kusel and Prue H. Hart

Pediatrics 2012;129;485; originally published online February 13, 2012;
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Maternal Serum Vitamin D Levels During Pregnancy and Offspring Neurocognitive Development

AUTHORS: Andrew J. O. Whitehouse, PhD, Barbara J. Holt, BSc, Michael Serralha, BSc(Hons), Patrick G. Holt, DSc, Merci M. H. Kusel, MBBS, and Prue E. Hart, PhD

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KEY WORDS
vitamin D, neurocognition, language impairment, behavioral problems, emotional problems, Raine study

ABBREVIATIONS
95% CI—95% confidence interval
CBCL—Child Behavior Checklist
OR—odds ratio
PPVT-R—Peabody Picture Vocabulary Test—Revised

Ms Kusel and Dr Hart contributed equally to this work.

Drs Whitehouse, Kusel, and Hart developed the hypotheses; Ms Holt, Mr Serralha, Dr Holt, and Dr Hart analyzed serum samples for 25(OH)-vitamin D concentrations; and Dr Whitehouse conducted the statistical analyses and wrote the main drafts of the manuscript. All authors contributed to the interpretation and discussion of the results and other sections of the manuscript.

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WHAT'S KNOWN ON THIS SUBJECT: Vitamin D levels in the general population have decreased considerably over the past decade. The implications of maternal vitamin D insufficiency during pregnancy for offspring neurocognitive development remain unclear.

WHAT THIS STUDY ADDS: Studying a large sample and using a prospective longitudinal design, this study demonstrates a link between maternal vitamin D insufficiency during pregnancy and offspring language impairment. There was no association with childhood behavioral or emotional problems.

abstract

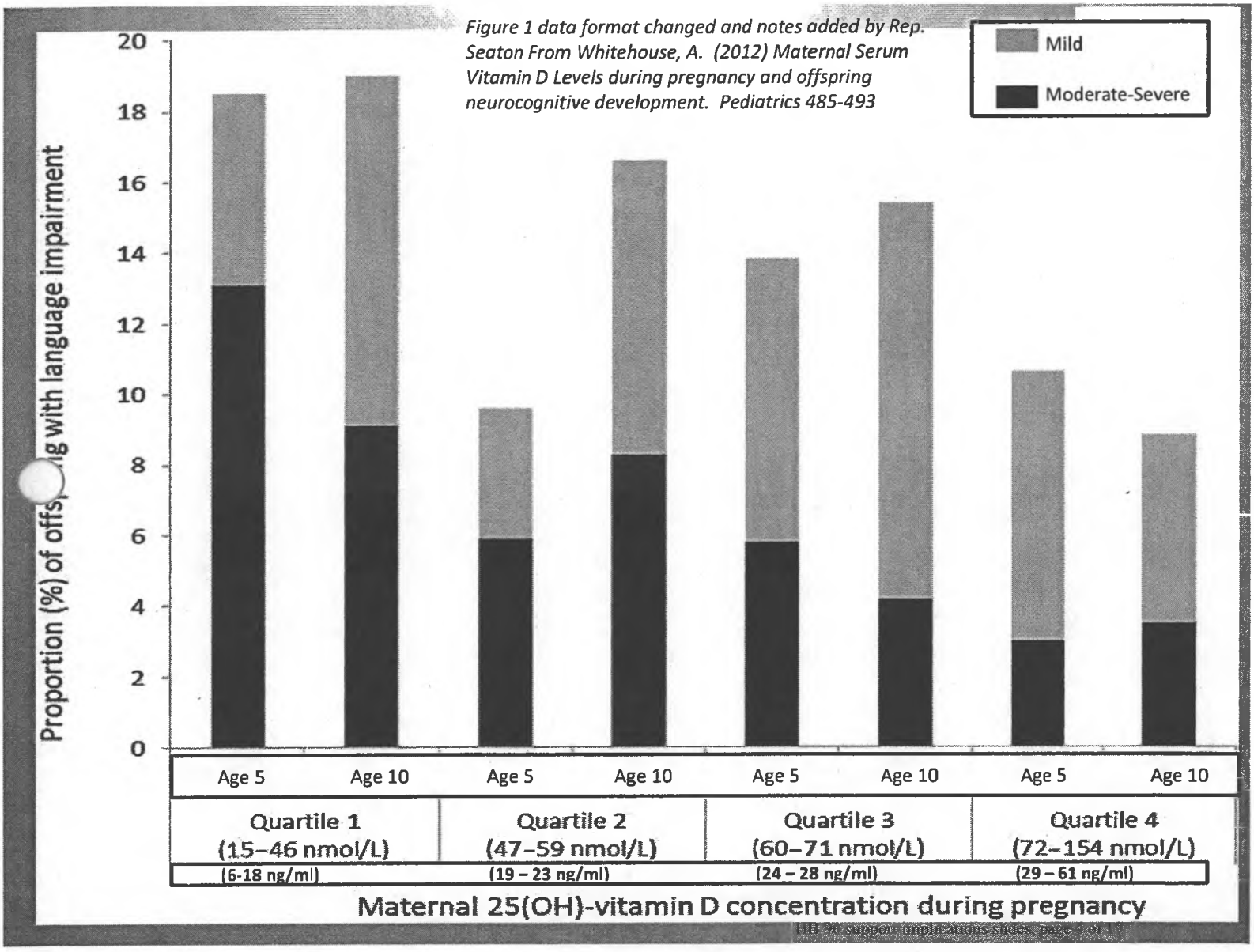
OBJECTIVE: To determine the association between maternal serum 25(OH)-vitamin D concentrations during a critical window of fetal neurodevelopment and behavioral, emotional, and language outcomes of offspring.

METHODS: Serum 25(OH)-vitamin D concentrations of 743 Caucasian women in Perth, Western Australia (32°S) were measured at 18 weeks' pregnancy and grouped into quartiles. Offspring behavior was measured with the Child Behavior Checklist at 2, 5, 8, 10, 14, and 17 years of age (n range = 412–652). Receptive language was assessed with the Peabody Picture Vocabulary Test—Revised at ages 5 (n = 534) and 10 (n = 474) years. Raw scores were converted to standardized scores, incorporating cutoffs for clinically significant levels of difficulty.

RESULTS: χ^2 analyses revealed no significant associations between maternal 25(OH)-vitamin D serum quartiles and offspring behavioral/emotional problems at any age. In contrast, there were significant linear trends between quartiles of maternal vitamin D levels and language impairment at 5 and 10 years of age. Multivariate regression analyses, incorporating a range of confounding variables, found that the risk of women with vitamin D insufficiency (≤48 nmol/L) during pregnancy having a child with clinically significant language difficulties was increased close to twofold compared with women with vitamin D levels >70 nmol/L.

CONCLUSIONS: Maternal vitamin D insufficiency during pregnancy is significantly associated with offspring language impairment. Maternal vitamin D supplementation during pregnancy may reduce the risk of developmental language difficulties among their children. *Pediatrics* 2012;129:485–493

Figure 1 data format changed and notes added by Rep. Seaton From Whitehouse, A. (2012) Maternal Serum Vitamin D Levels during pregnancy and offspring neurocognitive development. Pediatrics 485-493



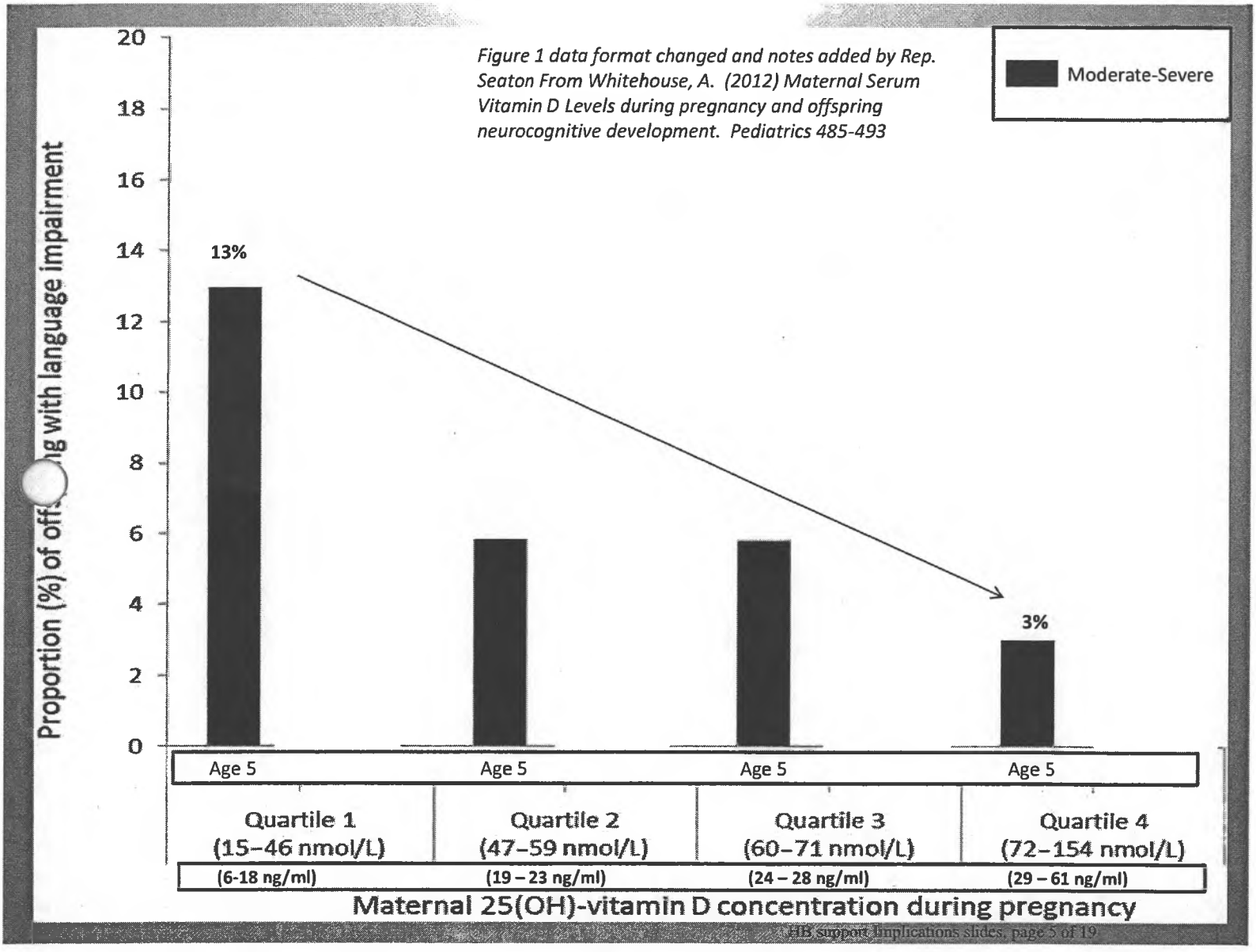
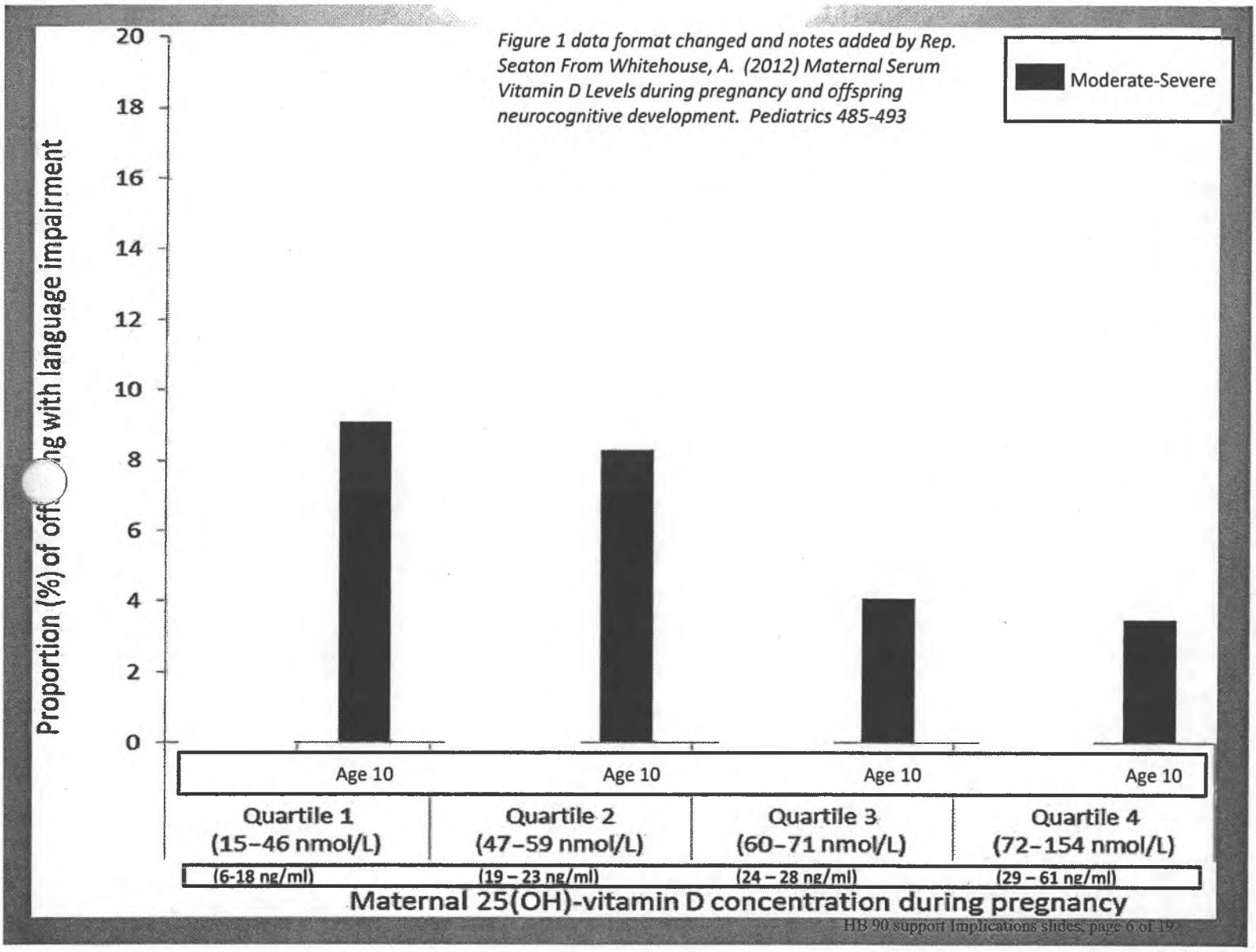


Figure 1 data format changed and notes added by Rep. Seaton From Whitehouse, A. (2012) Maternal Serum Vitamin D Levels during pregnancy and offspring neurocognitive development. Pediatrics 485-493
Moderate-Severe
Proportion (%) of off. ng with language impairment
20
18
16
14
12
10
8
6
4
2
0
13%
3%
Age 5
Age 5
Age 5
Age 5
Quartile 1 (15–46 nmol/L)
Quartile 2 (47–59 nmol/L)
Quartile 3 (60–71 nmol/L)
Quartile 4 (72–154 nmol/L)
(6-18 ng/ml)
(19 – 23 ng/ml)
(24 – 28 ng/ml)
(29 – 61 ng/ml)
Maternal 25(OH)-vitamin D concentration during pregnancy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Maternal 25(OH)-vitamin D concentration during pregnancy

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Spain

Mental and Psychomotor Development

Circulating 25-Hydroxyvitamin D_3 in Pregnancy and Infant Neuropsychological Development

Eva Morales, Mònica Guxens, Sabrina Llop, Clara L. Rodríguez-Bernal, Adonina Tardón, Isolina Riaño, Jesús Ibarluzea, Nerea Lertxundi, Mercedes Espada, Agueda Rodriguez and Jordi Sunyer

Pediatrics 2012;130;e913; originally published online September 17, 2012;
DOI: 10.1542/peds.2011-3289

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://pediatrics.aappublications.org/content/130/4/e913.1.full.html

Circulating 25-Hydroxyvitamin D_3 in Pregnancy and Infant Neuropsychological Development

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KEY WORDS
child development, cognition, infancy, intelligence, vitamin D

ABBREVIATIONS
CI—95% confidence intervals
FP—fractional polynomial
$25(OH)D_3$—25-hydroxyvitamin D_3

*Drs Morales and Guxens contributed equally to this work.

Dr Sunyer had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; Drs Morales, Guxens, Sunyer were responsible for study concept and design; Drs Guxens, Llop, Tardón, Ibarluzea, Espada, and Sunyer were responsible for acquisition of data; Drs Morales and Guxens were responsible for drafting of the manuscript; Drs Llop, Rodríguez-Bernal, Tardón, Riaño, Ibarluzea, Lertxundi, Espada, Rodríguez, and Sunyer were responsible for critical revision of the manuscript for important intellectual content; Dr Guxens was responsible for statistical analysis; and Drs Ibarluzea, Tardón, and Sunyer obtained funding.

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(Continued on last page)

WHAT'S KNOWN ON THIS SUBJECT: Adequate vitamin D status in mothers during pregnancy may influence the health status of offspring later in life. Growing evidence based on animal studies is linking vitamin D to brain development and functioning, but studies in humans are lacking.

WHAT THIS STUDY ADDS: This large-scale prospective pregnancy cohort study examines the association between maternal circulating 25-hydroxyvitamin D_3 concentrations in pregnancy and offspring neuropsychological development. Higher circulating concentration of 25-hydroxyvitamin D_3 in pregnancy was associated with improved mental and psychomotor development in infants.

OBJECTIVE: To investigate whether circulating 25-hydroxyvitamin D_3 [$25(OH)D_3$] concentration in pregnancy is associated with neuropsychological development in infants.

METHODS: The Spanish population-based cohort study Infancia y Medio Ambiente Project recruited pregnant women during the first trimester of pregnancy between November 2003 and February 2008. Completed data on 1820 mother-infant pairs were used. Maternal plasma $25(OH)D_3$ concentration was measured by high-performance liquid chromatography in pregnancy (mean 13.5±2.1 weeks of gestation). Offspring mental and psychomotor scores were assessed by trained psychologists at age 14 months (range, 11–23) by using the Bayley Scales of Infant Development. β-Coefficients with 95% confidence intervals (CIs) of mental and psychomotor scores associated with continuous or categorical concentrations of maternal plasma $25(OH)D_3$ were calculated by using linear regression analysis.

RESULTS: The median plasma value of $25(OH)D_3$ in pregnancy was 29.6 ng/mL (interquartile range, 21.8–37.5). A positive linear relationship was found between circulating concentrations of maternal $25(OH)D_3$ concentrations in pregnancy and mental and psychomotor scores in the offspring. After adjustment for potential confounders, infants of mothers with $25(OH)D_3$ concentrations in pregnancy >30 ng/mL showed higher mental score (β = 2.60; 95% CI 0.63–4.56) and higher psychomotor score (β = 2.32; 95% CI 0.36–4.28) in comparison with those of mothers with $25(OH)D_3$ concentrations <20 ng/mL.

CONCLUSIONS: Higher circulating concentration of maternal $25(OH)D_3$ in pregnancy was associated with improved mental and psychomotor development in infants. *Pediatrics* 2012;130:e913–e920

TABLE 1 Characteristics of Participants According to Maternal Circulating 25(OH)D_3 Concentrations in Pregnancy

	Serum 25(OH)D_3 Concentration			*P* Value Trend
	<20 ng/mL (*n* = 356)	20–30 ng/mL (*n* = 574)	>30 ng/mL (*n* = 890)	
Area of study				
Valencia (39°N latitude)	20.2	29.6	37.2	<.001
Sabadell (41°N latitude)	30.3	23.2	26.4	
Gipuzkoa (42°N latitude)	24.7	27.2	23.4	
Asturias (43°N latitude)	24.7		13.0	
Child's gender (male)	51.1		48.8	.722
Birth weight, g	3300 (435)		2 (419)	.685
Maternal			2 (4.0)	.004
Parity			44.3	.053
Mater			7.4	.460
Paren				
I/II			36.0	.037
III			26.5	
IV/			37.5	
Mater				
Pri			22.3	.273
Se			39.4	
Un			38.3	
Mater				
Nor			75.6	.086
Overweight		19.0	16.6	
Obese (≥3			7.8	
Smoking at t			13.9	.022
Alcohol during pregnancy (yes)	18.0	16.4	22.4	.013

Values are percentages for categorical variables and mean (SD) for continuous variables.

>30 ng/mL (*n* = 890)

Area of study
- Valencia (39°N latitude) → 37.2
- Sabadell (41°N latitude) → 26.4
- Gipuzkoa (42°N latitude) → 23.4
- Asturias (43°N latitude) → 13.0

Alaska (53° to 71° N latitude) → ?

HB 90 Support Implications, 9-19

A positive linear relationship was found between circulating concentrations of maternal 25(OH)D_3 in pregnancy and both mental (Fig 3A) and psychomotor (Fig 3B) development scores in the offspring. In multivariable models, each 10 ng/mL increase in 25(OH)D_3 in pregnancy resulted in up to 0.79 and 0.88 points increase in mental and psychomotor development scores in offspring, respectively (Table 2). In the basic model with adjustment for area of study, infants of mothers with 25(OH)D_3 concentrations >30 ng/mL showed an advantage of 3.17 and 2.42 points in the mental and psychomotor scores, respectively, in comparison with those of mothers with 25(OH)D_3 concentrations <20 ng/mL (model 1) (Table 2). Although attenuated, these associations remained significant after adjustment for potential confounders including child's gender, birth weight, maternal country of origin, maternal age, parental socioeconomic status, maternal education level, parity, maternal pre-pregnancy BMI, and

DISCUSSION

To our knowledge this is one of the first large-scale prospective pregnancy cohort studies to examine the association between maternal circulating 25(OH)D_3 concentrations in pregnancy and offspring neuropsychological development in infancy. Higher concentrations of circulating 25(OH)D_3 in pregnancy were associated with improved mental and psychomotor scores. Infants of mothers with 25(OH)D_3 concentrations >30 ng/mL (clinically considered as optimal levels) showed an advantage of 2.6 and 2.3 points in mental and psychomotor scores, respectively, in comparison with those of mothers with 25(OH)D_3 concentrations <20 ng/mL (considered as deficient levels). The association remained significant after adjusting for a wide range of potential confounding and intermediate factors.

The main strengths of this study include its population-based prospective design and large sample size as well as examination of the associations with

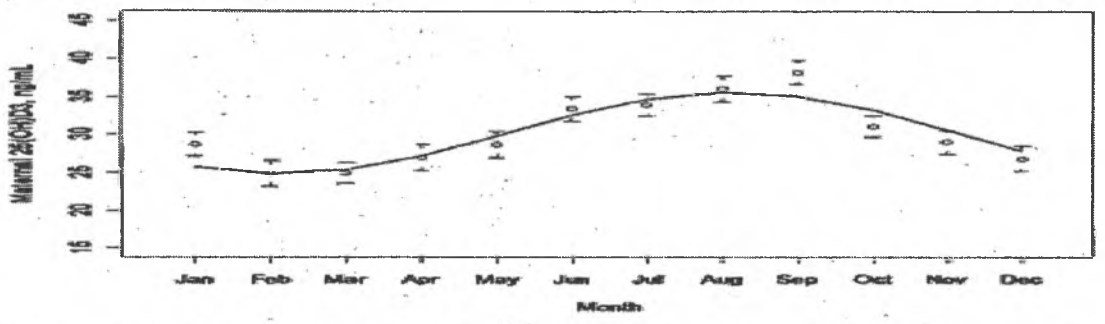

FIGURE 2
Fitted sinusoidal model for observed maternal circulating concentration of 25(OH)D_3 superimposed on plot of observed monthly mean and 95% CI values for 25(OH)D_3 concentration among 2112 participants in the INMA Project.

HB 90 support Implications, 10-19

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The Journal of Nutrition
Community and International Nutrition

High Prevalence of Vitamin D Insufficiency in Black and White Pregnant Women Residing in the Northern United States and Their Neonates[1]

Lisa M. Bodnar,[2–4]* Hyagriv N. Simhan,[2–4] Robert W. Powers,[3,4] Michael P. Frank,[4] Emily Cooperstein,[4] and James M. Roberts[2–5]

[2]Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA 15261; [3]Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213; and [4]Magee-Women's Research Institute, Pittsburgh, PA 15213

Abstract

In utero or early-life vitamin D deficiency is associated with skeletal problems, type 1 diabetes, and schizophrenia, but the prevalence of vitamin D deficiency in U.S. pregnant women is unexplored. We sought to assess vitamin D status of pregnant women and their neonates residing in Pittsburgh by race and season. Serum 25-hydroxyvitamin D [25(OH)D] was measured at 4–21 wk gestation and predelivery in 200 white and 200 black pregnant women and in cord blood of their neonates. Over 90% of women used prenatal vitamins. Women and neonates were classified as vitamin D deficient [25(OH)D <37.5 nmol/L], insufficient [25(OH)D 37.5–80 nmol/L] or sufficient [25(OH)D > 80 nmol/L]. At delivery, vitamin D deficiency and insufficiency occurred in 29.2% and 54.1% of black women and 45.6% and 46.8% black neonates, respectively. Five percent and 42.1% of white women and 0.7% and 56.4% of white neonates were vitamin D deficient and insufficient, respectively. Results were similar at <22 wk gestation. After adjustment for prepregnancy BMI and periconceptional multivitamin use, black women had a smaller mean increase in maternal 25(OH)D compared with white women from winter to summer (16.0 ± 3.8 nmol/L vs. 23.2 ± 3.7 nmol/L) and from spring to summer (13.2 ± 3.0 nmol/L vs. 27.6 ± 4.7 nmol/L) ($P < 0.01$). These results suggest that black and white pregnant women and neonates residing in the northern US are at high risk of vitamin D insufficiency, even when mothers are compliant with prenatal vitamins. Higher-dose supplementation is needed to improve maternal and neonatal vitamin D nutriture. J. Nutr. 137: 447–452, 2007.

Babies at birth deficient or insufficient = 92.4% = 66.1%

Introduction

Rickets, once thought to have been nearly eradicated in the United States in the 1930s (1), has again become a major public health problem. Several reports have been published describing recent cases of rickets in infants, most of whom were black and exclusively breastfed (2–5). The reemergence of rickets is thought to be due to an epidemic of vitamin D deficiency in mothers and children (6). A newborn's vitamin D stores are completely reliant on vitamin D from the mother (7). Not surprisingly, poor maternal vitamin D status during pregnancy is a major risk factor for infant rickets (8–10).

In addition to causing poor global mineralization of the skeleton, vitamin D deficiency has implications for numerous other nonskeletal health outcomes. In utero or early life vitamin D deficiency has been linked to an increased risk of type 1 diabetes (11), asthma (12), and schizophrenia (13,14). Fascinating new data also show that vitamin D regulates placental development and function (15), which suggests that maternal vitamin D status may be associated with adverse outcomes of pregnancy, such as miscarriage, preeclampsia, and preterm birth.

The most important source of vitamin D is the skin's synthesis of the vitamin from UV B solar radiation (16). Any process that reduces UV B photons from entering the epidermis will diminish cholecalciferol (vitamin D-3) production. The skin pigment melanin absorbs UV B photons and can reduce vitamin D-3 synthesis by >90% (17). Consequently, African Americans are at high risk of vitamin D deficiency. The most recent data from the National Health and Nutrition Examination Survey (1988–1994) indicated that vitamin D deficiency [25-hydroxyvitamin D [25(OH)D] ≤ 37.5 nmol/L] was prevalent in 42% of black childbearing-aged women and only 4% of white childbearing-aged women residing throughout the United States (18). Vitamin D status is also worsened in winter months (November through March), when, at latitudes above 37°, less UV B radiation reaches the earth and little or no vitamin D can be synthesized in the skin (16,19). Indeed, vitamin D deficiency in U.S. childbearing-aged women was more than 3 times as common in winter than summer in both blacks and whites (18).

Despite the striking racial disparity in vitamin D deficiency and the strong influence of season, there are few recent investigations into the vitamin D status of U.S. black and white pregnant women and their neonates throughout the year. Given

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* To whom correspondence should be addressed. E-mail: bodnar@edc.pitt.edu.

0022-3166/07 $8.00
Manuscript received 30 August 2006. Initial review completed 27 September 2006. Revision accepted 9 November 2006.

HB 90 support Implications, 11-19

TABLE 2 Vitamin D status of white and black pregnant women and their neonates[1]

	White women, $n = 200$	Black women, $n = 200$
4–21 wk gestation		
Serum 25(OH)D,[2] *nmol/L*	73.1 (69.4, 76.9)	40.2 (37.9, 42.7)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	2.0	44.9**
Insufficient: 25(OH)D 37.5–80 nmol/L	60.3	51.0
Sufficient: 25(OH)D >80 nmol/L	37.3	4.1
37–42 wk gestation		
Serum 25(OH)D, *nmol/L*	80.4 (76.0, 85.1)	49.4 (46.1, 52.9)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	5.0	29.2**
Insufficient: 25(OH)D 37.5–80 nmol/L	41.2	54.1
Sufficient: 25(OH)D >80 nmol/L	53.8	16.7
Cord blood		
Serum 25(OH)D, *nmol/L*	67.4 (63.8, 71.3)	39.0 (36.3, 41.8)*
Vitamin D status, %		
Deficient: 25(OH)D <37.5 nmol/L	9.7	45.6**
Insufficient: 25(OH)D 37.5–80 nmol/L	56.4	46.8
Sufficient: 25(OH)D >80 nmol/L	33.9	7.6

[1] Values are geometric means [95%CI] or %. *Different from white women, $P < 0.001$ (student's t test); **different from white women, $P < 0.001$ (chi-square test).
[2] Log-transformed to ensure normality.

HB support Implications, 12-19

Arch Dis Child. 2013 Jan 8. [Epub ahead of print]

Vitamin D status of exclusively breastfed infants aged 2-3 months.

Wall CR, Grant CC, Jones I.

Source: Discipline of Nutrition, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand.

Abstract

BACKGROUND: New Zealand in 2008 adopted WHO policy which recommends that all infants are exclusively breast fed until 6 months of age. The benefits of this policy for the infant are undisputed; however, this policy has the potential to adversely impact on infant vitamin D status. A number of countries now recommend that all breastfed infants receive daily vitamin D supplementation of 400 IU to prevent rickets. New Zealand has no policy on the vitamin D supplementation of 'low-risk' breastfed infants. There are no data on the vitamin D status of exclusively breastfed infants in the first few months of life in New Zealand.

AIM: To describe serum 25-hydroxy-vitamin D (25(OH)D) concentrations in exclusively breastfed infants aged 2-3 months.

DESIGN/METHODS: Healthy term exclusively breastfed infants who were receiving no vitamin D supplements were enrolled over a 15-month period. A capillary blood sample was obtained from each infant. Serum 25(OH)D was measured using isotope-dilution liquid chromatography-tandem mass spectrometry.

RESULTS: 94 infants were enrolled (mean age 10 weeks). Median 25(OH)D concentration was 53 nmol/l (IQR 14-100 nmol/l). 23 (24%) infants had serum 25(OH)D concentration <27.5 nmol/l. Infants enrolled during winter had a median (IQR) 25(OH)D serum concentration of 21 nmol/l (14,31). Infants enrolled during summer had a median (IQR) 25(OH)D concentration of 75 nmol/l (55 100) (winter vs summer, p<0.0001).

CONCLUSIONS: Vitamin D deficiency is prevalent in exclusively breastfed infants in New Zealand. Vitamin D supplementation should be considered as part of New Zealand's child health policy.

PMID:23303428[PubMed - as supplied by publisher]

Vitamin D levels of infants converted from nmol/l to the US common measurement for blood serum concentrations, ng/ml:

- -Entire study:
  - Median = 21.2 ng/ml
  - 24% of infants < 11 ng/ml
- -Infants enrolled in winter:
  - Median = 8.4 ng/ml
  - Highest level = 12.4 ng/ml
- -Infants enrolled in summer:
  - Median = 30 ng/ml

Conversion notes by the office of Representative Seaton

Alaska Newborn Vitamin D Assessment

- 10,000 babies
- 1 year
- $300,000

Your Support is Appreciated

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To: Representative Paul Seaton
State of Alaska Legislature

Re: Written Testimony in Support of HB 90

Date: 18 February 2013

Dear Representative Seaton,

As I am unable to attend the first hearing of HB 90 entitled "An Act establishing a temporary program in the Department of Health and Social Services for testing newborns for baseline vitamin D levels", I am hereby providing you with written testimony in support of HB 90.

I am a behavioral neuroscientist at the University of Alaska Fairbanks with research experience in animal behavior, behavioral genetics, biological rhythms, and compulsive-like behaviors. I also teach and have taught courses in animal physiology, neuroscience, and human anatomy and physiology.

I have great interest in vitamin D and its functions, as it has been implicated in many aspects of human health, which I stress in the human anatomy and physiology classes I teach. Vitamin D's traditional function has to do with absorption of calcium in the digestive tract and consequent bone health.[1] For example, deficiencies in vitamin D can result in reduced fetal bone growth[2], while treatment with vitamin D can cure rickets, a severe bone deficiency, in infants.[3]

The evidence that vitamin D plays important roles in other physiological processes in humans is accumulating rapidly.[4] For example, deficiencies in Vitamin D have been correlated with decreased immune function[5], hyper parathyroidism[6], and increased risks of cancer[7], dental caries[8], respiratory infections[9,10], eczema[11], lower birth weight and head circumference[12], and multiple sclerosis (MS).[13]

Many of these conditions are prevalent in Alaska.[14] In addition and directly relevant to HB 90, newborns have been found to be deficient in vitamin D from China[12], to Scotland[15] and the USA[16], potentially exposing them to higher risks for developing these conditions. These studies were done at lower latitudes than Alaska. Consequently, newborns in Alaska may have even more severe deficiencies in vitamin D due to sun exposure that is insufficient during 8-9 months of the year to produce vitamin D in the skin.

Importantly, vitamin D deficiency also has been correlated with decreased mental and psychomotor functions[17] and language impairment.[18] What if severe deficiencies in vitamin D might explain some of the academic impairments seen in Alaska's schools? Unfortunately, I am not aware of any studies that have measured vitamin D in Alaska newborns. Therefore, I strongly support your efforts to pass SB 90 during this legislative session to obtain crucial base line data on vitamin D levels in Alaska's newborns.

I hope that you will encourage your colleagues to ensure that Alaskans do the analysis of the data and that you will ask faculty at the University of Alaska to do this as a paid service to the State of Alaska.

Respectfully,

Abel Bult-Ito, Ph.D.
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For Paul Seaton

Re: HB90

Written testimony re: HB 90

As a long-time practicing physician in Homer, I would like to share my experience on the use of Vitamin D in a full scope "rural" family Practice over the last 7 years. I was first introduced to Vitamin D 8 years ago through the work of John Cannell, MD of the Vitamin D Council and have investigated many of his claims by reading some of the extensive scientific literature that he provides on his website, originating as early as the 1920s. I would also agree with his comments that we should truly consider Vitamin D a hormone, rather than a vitamin, based on its mechanism of action.

Our annual health fair began voluntary testing for Vitamin D several years ago and it was clear that the great majority of those tested were markedly deficient in vitamin D. While reviewing those results, I could correlate reported self-supplementation with Vitamin D with those results and it became clear that those that were supplementing at a level of 5000 IU a day were very likely to have an adequate level, with a declining dose dependent correlation. At that time we began to recommend that level of supplementation to all patients during their annual visits. In the years since, most patients have been very appreciative of that advice with anecdotal reports that they feel better, are less depressed and have had less respiratory infections in that time. From a personal standpoint, at the same level of supplementation, despite my exposure to every illness in the community, I have had much less lost time to illness than in previous years. This has been independently confirmed by a community Health Aide in Northwest Alaska who has reported a marked decrease in community illness with village supplementation of Vitamin D.

Our next step was to begin supplementation of Vitamin D to all our pregnant patients at a level of 5000 units a day. We believe in this so strongly, given all the new clinical associations of beneficial neonatal outcomes, we provide the Vitamin D for free to our patients. I can remember, years ago, being taught that Vitamin D was not available in breast milk and that babies could get all the Vitamin D they needed from 20 minutes of exposure of their cheeks to the sun each day. It now turns out that the reason there is no Vitamin D in breast milk is because most women are severely deficient in their levels, and that babies need more than just Alaskan sun exposure to get to the appropriate new levels, with the subsequent benefits alluded to above. We are now asking all parents to supplement their children of any age.

As this information I have presented is all truly anecdotal, I very much welcome the chance to see the results of HB 90 and actually quantitate the Vitamin D levels in our newborns. Assuming that the suppositions we are making about Vitamin D will continue to be borne out, and they currently seem to

be, the administration of Vitamin D to our children, at appropriate levels could well turn out to be one of the most critical and cost effective interventions that we can make in this state.

William H Bell MD

Homer, AK.

Taneeka Hansen

From: Heaney, Robert P. <rpheaney@creighton.edu>
Sent: Thursday, February 07, 2013 11:23 AM
To: Rep. Paul Seaton
Cc: Taneeka Hansen
Subject: HB 90

Categories: Vitamin D

Dear Representative Seaton:

This is to indicate my enthusiastic support for HB 90, which you have introduced into the Twenty-eighth Legislature – First Session. It should provide accurate information on the vitamin D status of newborns in Alaska, a necessary first step in defining whether there is a problem of inadequate vitamin D status, and if so, what its magnitude may be.

The first year of life is a critical period for newborns, as many of the body systems are being programmed at that time for their function throughout life. There is a large body of persuasive evidence indicating that low vitamin D status during this period can have ramifications on the body's immune system, the development of chronic diseases such as diabetes, learning and language disabilities, and even serious mental disorders such as schizophrenia.

The bill wisely restricts the program to a single year, which should be sufficient to allow the State to define its vitamin D status, while keeping the costs relatively low.

Please call on me if I can provide further information or support. I truly believe that Alaska is setting the pace for the other 49 states, and I heartily applaud this effort.

Best regards,

Robert P. Heaney, M.D.

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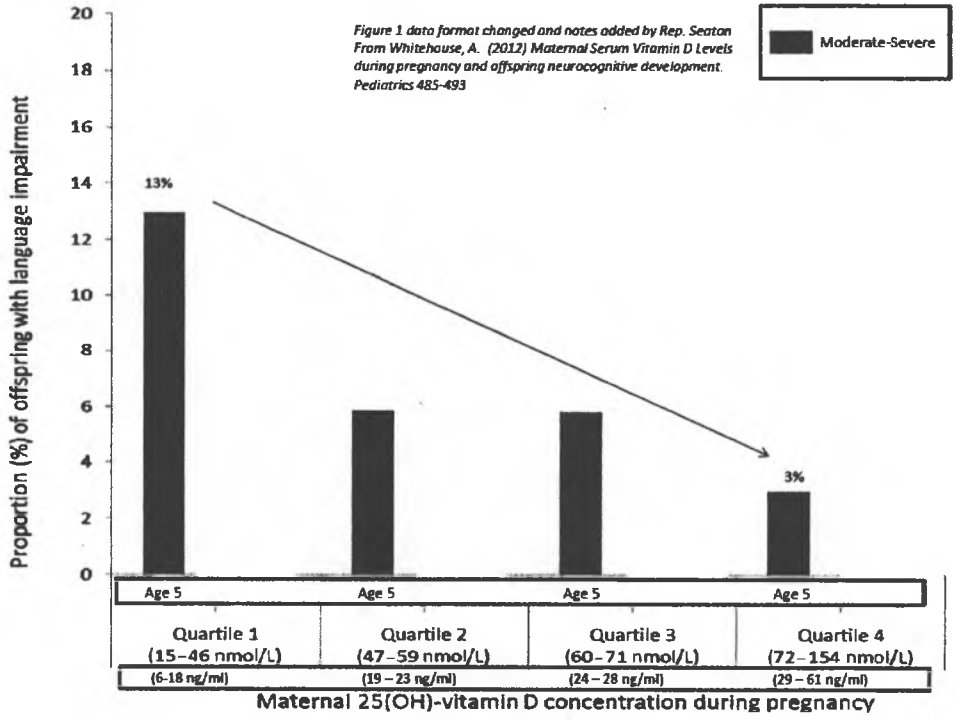
POTENTIAL IMPLICATIONS FOR ALASKA

If we assume that Alaska has the same distribution of vitamin D levels as Perth, Australia....

And if every child was then raised to the level of Quartile 4 (29-61 ng/ml) where the rate of moderate-severe language impairment is approximately 3%...

Then, from an Alaskan birth population of 10,000 a year, raising the vitamin D level to above 30 ng/ml could mean 400 fewer children *per year* entering kindergarten with moderate to severe language impairment.



THE STATE of ALASKA
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January 29, 2013

Analysis of Vitamin D Supplementation Studies

The first part of this paper will address the study related to military suicides and vitamin D that was noted in the House Finance Budget Subcommittee for Health and Social Services hearing on January 22, 2013. The second part will address other studies and the overall issue of the potential benefits of Vitamin D supplementation.

Part 1. Vitamin D and Suicide

Dr. Ward Hurlburt, Chief Medical Officer, reviewed the article in question. On January 4, 2013 a study titled "Low Vitamin D Status and Suicide: A Case-Control Study of Active Duty Military Service Members" was published in PLOS ONE. As noted in the title, this report is of a case-control type study. This means that a cohort of individuals who had experienced the outcome (completed suicide in this case) are compared to a group who had not experienced this outcome. Case-control studies, while not providing as high grade evidence as a prospective blinded randomized comparison, do often provide useful information and have the potential to be more reliable than some other kinds of evidence, such as expert opinion or expert consensus conferences. We appreciate this study being brought to our attention.

POLS ONE is one of the newer on line journals that seeks to publish relevant research in a timely manner and provides an opportunity for peer review of the submitted articles. PLOS ONE is an open-access journal and authors pay a fee to have their articles published. The lead author of the study was John C. Umhau, MD, a Senior Clinical Investigator at the National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health. No conflicts of interest were disclosed, but the authors noted that Dr. John Cannell provided insightful discussions regarding vitamin D. While not listed as an author of the article, Dr. Cannell is one of the early and active leaders advocating for the benefits of vitamin D supplementation. Separately in a review Dr. Cannell stated that Dr. Umhau has been engaged with the vitamin D advocacy community since 2007.

As is typical of a case-control type study there was and normally is an attempt to match the two groups in terms of age, gender, and other factors (including rank in this study). The two groups contained 495 individuals each. The suicide group was those who committed suicide between

2002 and 2008 and who had had their blood sampled within 24 months of death. A sample of plasma for the individuals in the comparison groups had been maintained by the Department of Defense and was available for analysis for vitamin D levels.

The individuals in this study were divided into "octiles" based on the vitamin D level of their stored plasma. The lowest "octile" had vitamin D levels of less than 15.5ng/ml. The highest "octile" had vitamin D levels above 36 ng/ml. The number of those with completed suicides in each "octile" was compared to the number in the control group. There was a statistically significant difference in the ratio of those who had committed suicide in the lowest "octile" compared to the aggregate of the other seven "octiles".

The selection of these "octiles" and the levels of plasma vitamin D are at the discretion of the author. This does not reflect a common practice in the scientific community. One of the things one looks for in analyzing a scientific article is how the authors break their groups down. Creative manipulation of grouping of study subjects can facilitate apparent support for desired results. With the authors having selected an "octile" breakdown it is of interest that there is not a significant difference between the completed suicide rate for "octile one" (the lowest vitamin D levels) and "octile six" (the third highest vitamin D levels). There would be little disagreement that a vitamin D level of < 15.5ng/ml is on the low side. It is of interest that Figure 1 of the article demonstrates no progression of reduction in completed suicide rate with increasing "octile" of the comparison group. The eighth (highest) "octile" has a suicide rate just mid range of octiles two through seven. In the comparison of the 495 suicide completers and the 495 individuals in the control group, the average plasma vitamin D level was almost identical (24.5 ng/ml in the suicide group and 24.8 ng/ml in the control group), as noted in Table 1 of the article.

As described above, the 990 individuals studied were broken down into "octiles" with the eighth octile being those with a plasma vitamin D of more than 36 ng/ml. GrassrootsHealth is "A Consortium of Scientists, Institutions, and Individuals Committed to Solving the Worldwide Vitamin D Deficiency Epidemic". They state: "The important thing is to achieve and maintain a range of 40 – 60 ng/ml" of plasma vitamin D.

We appreciate this study being brought to our attention. We believe the methodology used to analyze the data is flawed and that the lack of differences in Vitamin D levels between the suicide and control groups does not suggest an association between Vitamin D deficiency and suicide – much less a causal relationship.

Part 2. Other Vitamin D Studies

Many articles continue to be published related to vitamin D, a few of which committee members have shared with the Department of Health and Social Services. Two years ago the department had the opportunity to review both a text supporting the claims for widespread benefits to be derived from high doses of vitamin D supplementation and a flash drive from a conference held

at UCSD that was shared by a committee member. Presenters claimed that 99% of colon cancer and 80% of breast cancer could be prevented by having plasma vitamin D levels of 40 – 50 ng/ml. A wide array of other perceived potential benefits included dramatic reductions in the incidence of multiple sclerosis, diabetes, other cancers, cardiovascular disease, hypertension, toxemia of pregnancy – and many more. There is general consensus that vitamin D deficiency is related to rickets, osteomalacia, fractures and falls in the elderly, and some uncommon genetically determined bone diseases. There is disagreement regarding what is a "normal" serum vitamin D level and what is an appropriate supplemental dose.

Below are some quotes from entities such as the Institute of Medicine and its taskforces, the US Preventive Services Task Force, The Agency for Healthcare Research and Quality, the National Institutes of Health, and private entities that evaluate the quality of evidence such as the Cochrane Collaborative and Hayes.

1. Agency for Healthcare Research and Quality
    a. "Analyses of Third National Health and Nutrition Survey (NHANES III) showed no significant association between baseline serum 25(OH)D concentrations and total cancer mortality".
    b. "..no significant association between serum 25(OH)D concentrations and cardiovascular death, myocardial infarction, or stroke".
2. Institute of Medicine
    a. From Harvard Health Publications – the conclusion of the four page summary of the book length report – "Scientific evidence indicates that calcium and vitamin D play key roles in bone health. The current evidence, however, does not support other benefits for vitamin D or calcium intake. However, the committee emphasizes that with few exceptions all North Americans are receiving enough calcium and vitamin D. Higher levels have not been shown to confer greater benefits, and in fact, they have been linked to other health problems, challenging the concept the 'more is better'".
    b. "Vitamin D proponents have also said the goal for blood levels should be 30ng/ml. The IOM panel says levels that high are not associated with any health benefit and ads that levels above 50ng/ml 'may be reason for concern'".
3. National Institutes of Health (Office of Dietary Supplements)
    a. "Perhaps surprisingly, geographic latitude does not consistently predict average serum 25(OH)D levels in a population. Ample opportunities exist to form vitamin D (and store it in the liver and fat) from exposure to sunlight during spring, summer, and fall months even in the far north latitudes".
    b. "Taken together, however, studies to date do not support a role for vitamin D, with or without calcium, in reducing the risk of cancer".
    c. "One meta-analysis found use of vitamin D to be associated with a statistically significant reduction in overall mortality from any cause, but a reanalysis of the data found no association.
    d. "Vitamin D toxicity can cause non-specific symptoms such as anorexia, weight loss, polyuria, and heart arrhythmias. More seriously it can also raise blood levels of calcium which leads to vascular and tissue calcification, with subsequent

damage to the heart, blood vessels, and kidneys". "...concluded that serum 25(OH)D levels above 50 – 60 ng/ml should be avoided, as even lower serum levels of 30 – 48 ng/ml are associated with increases in an all cause mortality, greater risk of cancer at some sites like the pancreas, greater risk of cardiovascular events, and more falls and fractures among the elderly".

4. Cochrane Summary
    a. "The review authors suggest that until further high level evidence is available, clinicians should continue to follow local guidelines in people with MS. However, the question of safety and effectiveness of vitamin D in people of MS remains unanswered".
5. U S Preventive Services Task Force (From draft recommendations related to Vitamin D and Calcium Supplementation to Prevent Cancer and Osteoporotic Fractures in Adults :...")
    a. "During the 7 years of the trial, total cancer incidence and cancer mortality did not significantly differ between women in the intervention and placebo groups. Incidence and mortality rates of colorectal and invasive breast cancer ..... there were no statistically significant differences between intervention and control group rates of these types of cancer".
    b. The summary of the draft recommendation from the USPSTF is that there is insufficient (I) evidence to assess the benefits and harms of vitamin D supplementation.... for the primary prevention of cancer in adults. The summary goes on to say in a "D" recommendation that they recommend against daily supplementation with 400 IU or less of vitamin D and 1000 mg calcium for the primary prevention of fractures in non-institutionalized postmenopausal women.
    c. In a separate communication the USPSTF recommends that vitamin D is effective in preventing falls in community dwelling adults aged 65 or older who are at increased risk for falls (B recommendation).

What these reports indicate is that the vast number of Vitamin D studies analyzed by these agencies do not support the value of higher vitamin D levels in the pathophysiology of disease states outside of bone health. Observational studies such as the ones referenced here are valuable for directing future research priorities, but not for establishing causation. One of the important reasons for this is that observational studies are, by design, subject to a number of important biases and other limitations. It is important to be vigilant not to overstate the science in any way. The Department is interested to learn more of vitamin D research findings over time, and we look forward to future collaboration with the Legislature on this important matter.

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ORIGINAL RESEARCH

Vitamin D and Pregnancy: Skeletal Effects, Nonskeletal Effects, and Birth Outcomes

Bruce W. Hollis · Carol L. Wagner

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Abstract The function and requirement of vitamin D during pregnancy for both mother and fetus have remained a mystery. This fact was highlighted by *The Cochrane Review* in 2000, which reported a lack of randomized controlled trials (RCTs) with respect to vitamin D requirements during pregnancy. Unfortunately, during the past decade only a single RCT has been performed with respect to vitamin D requirements during pregnancy. In this review we will discuss vitamin D metabolism during pregnancy as well as the consequences of vitamin D deficiency on skeletal, nonskeletal, and birth outcomes using birth observational data and data from our recent RCT. New RCT data strongly support previous observational studies in that improving nutritional vitamin D status will improve birth outcomes. The new RCT data indicate that 4,000 IU/day vitamin D_3 during pregnancy will "normalize" vitamin D metabolism and improve birth outcomes including primary cesarean section and comorbidities of pregnancy with no risk of side effects.

Keywords Vitamin D · Bone · Pregnancy · Birth outcome · Infant · 25-Hydroxyvitamin D

The current dietary recommendation for vitamin D during pregnancy remains archaic for a simple reason: fear of vitamin D toxicity [1]. To understand this statement, it is important to look at the history surrounding vitamin D during pregnancy. In 1947, Dr. E. Obermer [2] presented evidence that pregnant women required several thousand international units (IUs) of vitamin D daily during pregnancy. This recommendation had barely "seen the light of day" before vitamin D was erroneously associated with causing supravalvular aortic stenosis syndrome during pregnancy [3–6]. Thus, vitamin D was viewed as teratogenic to the developing fetus during pregnancy, and in response, the medical profession adhered to the largely insignificant 200 IU/day dosing recommendation for adults put forth by the Forbes committee in 1963 [7]. Sadly, at present, a similarly low recommendation largely remains in force as highlighted in the recent Institute of Medicine (IOM) document [8], although a more recent recommendation by the Endocrine Society, recommends higher dosing that takes into account the emerging data surrounding vitamin D's effect on nonskeletal functions [9]. It should be noted that the IOM report is a guide for food manufacturers, while the Endocrine Society report is a guide for the clinical care of patients.

Why are the recommendations between the IOM report and the Endocrine Society report so divergent? The answer is simple: the IOM report refused to make any recommendation not based on a randomized controlled trial (RCT), while the Endocrine Society used a vast combination of all available data including observational trials [9]. In this review, we investigate all avenues of data to derive conclusions.

What Constitutes a "Normal" Level of Circulating 25-Hydroxyvitamin D during Pregnancy?

If we go way back to human origins in Africa, vitamin D would never be considered a nutrient. During almost all of

B. W. H. is an academic consultant to Diasorin. The authors have stated that they have no other conflict of interest.

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human evolution, vitamin D was derived from solar exposure of the skin. Our human ancestors migrated out of Africa approximately 50,000 years ago to more northern latitudes, and thus, the intensity and duration of solar exposure decreased. A critical point of solar vitamin D generation occurred about 10,000 years ago in Europe when a gene controlling skin pigmentation mutated. This mutation decreased human skin pigmentation and allowed more efficient use of the limited sun exposure available at these northern latitudes [10]. If we progress ahead to the present, we are faced with clothing and other civilized customs that restrict solar exposure of the skin. Thus, now vitamin D truly becomes a vitamin because almost all of us now must obtain it from our diet. There is only one minor problem: vitamin D really does not exist in our food supply to any significant degree. As a result, we now must rely on supplementation to achieve our needs, and herein lies the problem.

Until the 1990s, the criterion for appropriate vitamin D nutrition was simply the absence of overt rickets or osteomalacia [7]. To deal with this problem, the IOM in 1997 recommended that infants and adults receive 400 and 200 IU/day vitamin D, respectively [11]. That dose eliminated rickets in children, but the effect of the adult dose remains a mystery as we do know that a 200 IU/day dose will not increase circulating 25-hydroxyvitamin D (25[OH]D) in adults [12]. Remember, in 1963 we had no idea that vitamin D underwent further metabolism in the human body. As it was discovered that vitamin D was metabolized to more active compounds in the late 1960s, important information began to emerge [13, 14].

In 1971, Haddad and Chyu [15] discovered that 25(OH)D circulated in "normal" adults at about 68 nmol. "Normal" for this study was described as anyone not demonstrating an overt affliction. This study also measured 25(OH)D levels in lifeguards, whose levels were shown to be approximately 175 nmol [15]. So, why not define the lifeguards as "normal" for circulating 25(OH)D and try to achieve those circulating levels of 25(OH)D in other humans? The answer is quite simple: 8 years earlier, the Blumberg report recommended only 200 IU/day vitamin D [7]; since 200 IU/day will put humans nowhere near 175 nmol circulating 25(OH)D, nature must be wrong. In 1971, we did not know how much vitamin D_3 was produced by solar exposure; however, that data were forthcoming [16]. As it turns out, unencumbered natural sun exposure to humans can generate thousands of IUs of vitamin D_3 in a short period of time [17]. Of course, these data did not agree with the Blumberg report of 1963, so their relevance was largely ignored.

The misinformation about vitamin D dosing in humans was just beginning. As mentioned earlier, in 1997, the IOM released a report that reaffirmed the vitamin D requirement for adults as 200 IU/day [11]. Worse, that committee also stated that humans can consume only 2,000 IU/day vitamin D for toxicity reasons [11]. Forget the fact that nature allows us to naturally produce 10,000–20,000 IU/day vitamin D_3 from modest solar exposure [17]. Again, nature must be in error. Thus, not only did medical societies accept that low circulating levels of 25(OH)D were "normal" but the premise was that we now "know" that even modest levels of dietary vitamin D, by natural synthesis standards, can harm you. So what is the truth?

To establish a more encompassing answer about "normal" human levels of circulating 25(OH)D or true vitamin D requirements, let us forget for a moment about the original 1963 Blumberg report [7] with respect to adults and the 1997 IOM toxicity report [11]. Let us return to the levels of circulating 25(OH)D in humans living in their original environment—tribal Africa. We have known for decades that sun-exposed humans in North America can attain high (250 nmol) circulating 25(OH)D [15]. We have also known for decades that nonhuman primates living in the wild possess circulating 25(OH)D levels up to 1,250 nmol/L [18]. Yet, this last point has been dismissed because they are not human. How about tribal, nomadic Africans? A new publication and data provide us clear answers to this question [19] (M. Luxwolda, personal communication).

Luxwolda et al. [19] clearly demonstrated that native tribal Africans achieve an average circulating 25(OH)D level of 115 nmol. But how about during pregnancy? Surely, nature would not allow such levels to occur since the recent IOM report states that such levels would be dangerous to the mother and fetus [8]. It appears that nature did not adhere to the IOM report because the recent study data demonstrates that pregnant women from different native tribes in Africa achieve an average circulating 25(OH)D level of 150 nmol/L throughout pregnancy. How do these natural levels compare with levels of 25(OH)D attained in oral supplementation studies in northern latitudes? In our recent RCT, pregnant women receiving 4,000 IU/day vitamin D_3 attained an average circulating 25(OH)D level of 111 nmol [1], well below the 150 nmol/L in a natural environment (M. Luxwolda, personal communication). This means that in a natural state, in which humans have evolved for more than 1 million years, nature has supplied many times the daily vitamin D recommended by Blumberg et al. [7] or the IOM [8, 11]. Our conclusion is that during pregnancy women should have a circulating 25(OH)D level in excess of 100 nmol/L; however, one achieves it, be it solar exposure and/or diet.

Finally, a comment on the upper safe intake limit (UL) for vitamin D during pregnancy, or anyone for that matter, deserves some attention. The practice of perpetuating a scientific myth has no better example than the toxicity

"myth" of vitamin D. This process for vitamin D has been eloquently documented by Vieth [12]; however, one particular point deserves to be highlighted for the harm it has caused. In 1997, the IOM committee on vitamin D and calcium decided to set a UL for vitamin D [11]. The intent was noble, but the result was a disaster because it was based on a single obscure study that today is regarded as invalid [20]. This resulted in setting the UL for vitamin D at 2,000 IU/day, which crippled clinical vitamin D trials for more than a decade. Why? It is because institutional review boards (IRBs) would not approve vitamin D studies that exceeded that intake. For example, to conduct our vitamin D pregnancy and lactation trials in 2003 [1], we had to write a full investigational drug application to obtain an investigational new drug (IND) number from the U.S. Food and Drug Administration (FDA). One would think that back in 1997 the IOM committee would have asked the following question: if humans can endogenously produce 10,000–20,000 IU vitamin D_3/day from natural sun exposure, how can an oral dose of 2,000 IU/day be of any harm? This same question should also have occurred to the 2010 IOM committee when they set the UL at 4,000 IU/day [8]. There is hope, however, because the 2011 Endocrine Society recommendation apparently did take all this into account when they assigned a 10,000 IU/day UL [9].

RCT of Vitamin D Supplementation during Pregnancy

Table 1 lists all of the RCTs with respect to vitamin D supplementation during pregnancy. There are some stark realities here. First, all but one was performed more than 25 years ago, primarily using vitamin D_2 as a supplement. Second, except for the Hollis et al. [1] study, all the trials used supplemental levels of vitamin D that had limited impact on rising circulating 25(OH)D levels. Finally, all the studies except that of Hollis et al. [1] were small and collected limited data; thus, conclusions are very difficult to extract.

A brief history of the older studies is described here. Initial vitamin D supplementation studies during pregnancy were carried out in the early 1980s. Brooke et al. [21], who studied British mothers of Asian descent, found a greater incidence of small-for-gestational-age (SGA) infants born to mothers who received placebo than for mothers who received 1,000 IU (25 μg) vitamin D_2/day during the final trimester of pregnancy. Neonates in the placebo group also had a greater fontanelle area than did the supplemented group. It must be noted that the placebo group in this study showed profound hypovitaminosis D. Follow-up studies by Brooke et al. [22] were conducted in Asian mothers who again were provided with either placebo or 1,000 IU

Table 1 Summary of RCT vitamin D supplementation studies during pregnancy

Reference	Number of subjects	Vitamin D dose (IU/day)	Therapy duration (months)	Initial 25(OH)D (nmol/L)	End-point 25(OH)D (nmol/L)
Brooke et al. [21][a]	67 Control	0	3	16.3	–
	59 Supplemented	1,000 D_2	3	20.0	168.0
Cockburn et al. [23]	82 Control	0	4	32.5	–
	82 Supplemented	400 D_2	4	39.0	42.8
Brooke et al. [22]	67 Control	0	3	–	–
	59 Supplemented	1,000 D_2	3	–	–
Maxwell et al. [24]	67 Control	0	3	–	–
	59 Supplemented	1,000 D_2	3	20.0	–
Marya et al. [96]	75 Control	0	3	–	–
	25 Supplemented	1,200 D_2	3	–	–
Delvin et al. [97]	15 Control	0	3	17.5 (cord)	–
	15 Supplemented	1,000 D_3	3	–	45.0 (cord)
Mallet et al. [28]	27 Control	0	3	9.5	–
	21 Supplemented	1,000 D_2	3	–	25.3
Hollis et al. [1][b]	111 Supplemented	400 D_3	6	61.5	79.0
	122 Supplemented	2,000 D_3	6	58.3	98.3
	117 Supplemented	4,000 D_3	6	58.3	111.0

[a] 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/day vitamin D_3 for 3 months [22]

[b] 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 United States 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 United States today with 0 IU vitamin D/day. Therefore, 400 IU vitamin D/day is the control group

vitamin D_2/day during the last trimester of pregnancy. The follow-up data provided evidence that, during the first year of life, the infants of the maternal placebo group gained less weight and had a lower rate of linear growth than did the infants of the maternal supplemented group.

Cockburn et al. [23] undertook a large vitamin D supplementation study of >1,000 pregnant subjects in the United Kingdom who were supplemented with 400 IU (10 μg) vitamin D_2/day or received a placebo from week 12 of gestation onward. At this level of supplementation, serum concentrations of 25(OH)D in the supplemented group were only slightly higher than those in the placebo group. A defect in dental enamel formation was observed in a higher proportion of the children at 3 years of age in the maternal placebo group. Maxwell et al. [24] conducted a double-blind trial of vitamin D (1,000 IU/day) during the last trimester of pregnancy in Asian women living in London. They found that the supplemented mothers had greater weight gain and, at term, had significantly higher plasma concentrations of retinol-binding protein and thyroid-binding prealbumin, which indicated better protein-calorie nutrition. Almost twice as many infants of the unsupplemented group weighed <2,500 g at birth (the definition of low birth weight) and had significantly lower retinol-binding protein concentrations than did infants of the supplemented mothers.

Supplementation with 1,000 IU (25 μg) vitamin D/day during the last trimester of pregnancy has produced mixed results. The initial study by Brooke et al. [22] described a dramatic increase, from 125 to 150 nmol/L in circulating 25(OH)D, in both mothers and neonates at term. However, these results are highly suspect in light of later and current work and are consistent with a dose response obtained after consumption of 10,000 IU (250 μg) vitamin D/day for 3 months [25]. There also is the possibility that the 25(OH)D assay method used in this study was flawed, as was common during this early period of investigation [26, 27]. Consistent with more recent data, Mallet et al. [28] reported that vitamin D supplementation (1,000 IU/day or 25 μg/day) during the last trimester of pregnancy resulted in an increase in circulating 25(OH)D concentrations of only 12.5–15 nmol/L in maternal and cord serum.

As concluded by *The Cochrane Review* in 2000 [29], these early studies provided an insufficient basis to make any recommendations about vitamin D supplementation during pregnancy. As a result, in 2004, our laboratory initiated a National Institute of Child Health and Human Development–sponsored 6 year randomized, double-blind, placebo-controlled trial of vitamin D supplementation during pregnancy to assess safety and pregnancy outcomes with an approved investigational drug application from the FDA (66,346). The logical question is why it took 25 years before this trial was performed, and the answer is twofold. First, it was expensive, costing about $6 million to perform. Second, until we wrote and received the IND from the FDA, the IRB at our university (representative of other IRBs) would not allow the study to commence, fearing we would do harm by administering the amount of vitamin D (4,000 IU/day) we were proposing. Once the IND was in place, the study could be conducted. The results of our completed study have been published [1] and will be discussed in detail below.

In reference to more recent publications, one of the most puzzling developments was the omission of our RCT pregnancy study from the 2012 Cochrane review of vitamin D supplementation for women during pregnancy [30]. One of us (C. L. W.) was invited to present our published RCT results to the World Health Organization in the fall of 2011. She was informed that the study would not be considered in the upcoming Cochrane review because we did not have a "control" group, meaning a group of pregnant women receiving 0 IU vitamin D during pregnancy. Such a group would be unethical in many regions of the world today because it violates the "standard of care" of giving 400 IU vitamin D/day as part of the prenatal vitamin. Zero IU dosing of vitamin D to those women therefore would never be approved by any knowledgeable IRB in those regions of the world, including the United States, Canada, and most of Europe. For this reason, the applicability of the latest Cochrane review to a major sector of the world is limited.

Vitamin D Metabolism during Pregnancy

Vitamin D metabolism during pregnancy is vastly different from any other time in human physiology, and this point has gone largely unappreciated. With respect to the conversion of vitamin D to 25(OH)D, this metabolic conversion appears to be similar in pregnant and nonpregnant states and follows first- and zero-order enzyme kinetics (Fig. 1) [1, 31]. The similarities, however, end there. It has been known for decades that during pregnancy 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) levels become extremely elevated [32–34]. This increase in circulating $1,25(OH)_2D$ levels has in particular been attributed to an increase in the serum vitamin D-binding protein (DBP) that would regulate the amount of "free" $1,25(OH)_2D$ available in the circulation [33]. While this rise in DBP during pregnancy has been shown to be 46–103 %, depending on the assay employed [35], it cannot account for the nearly three- to-fourfold increase in circulating $1,25(OH)_2D$ in our recent study [1]. Bikle et al. [34] clearly demonstrated that free $1,25(OH)_2D$ levels are increased during pregnancy despite the significant increase in DBP levels, and our recent data agree with this premise [1]. In fact, the new data from our

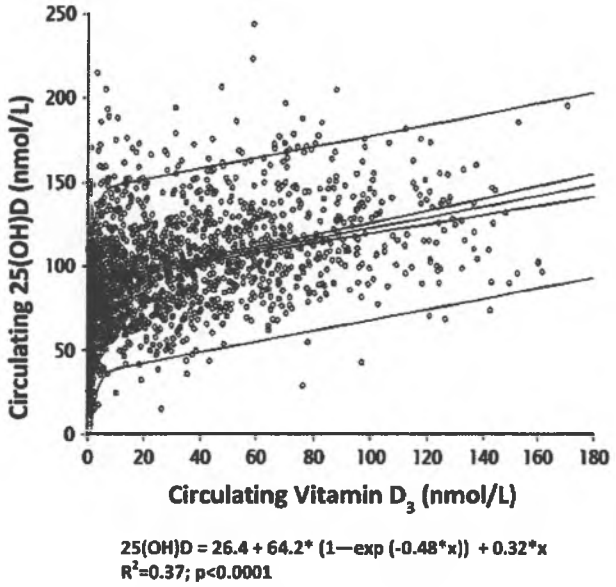

Fig. 1 The relationship between circulating vitamin D to control the production of 25(OH)D during pregnancy [1]

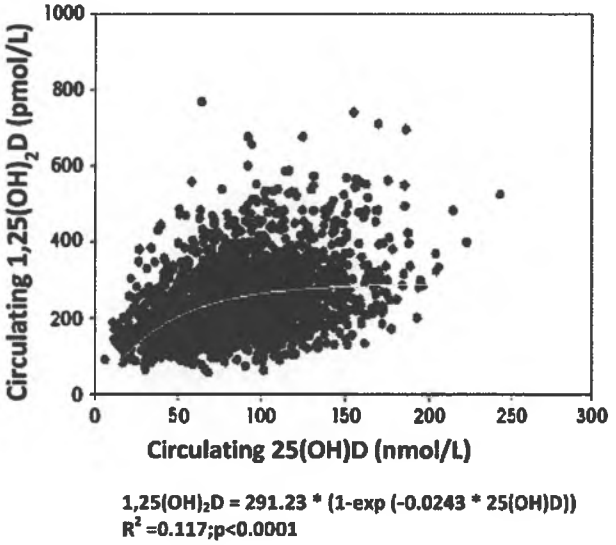

Fig. 2 Relationship of circulating 25(OH)D to circulating $1,25(OH)_2D$ during pregnancy [1]

study demonstrate that a circulating 25(OH)D level of approximately 40 ng/mL (100 nmol/L) is required to optimize production of $1,25(OH)_2D$ during human pregnancy through renal and/or placental production of the hormone (Fig. 2) [1]. Again, this relationship exhibits first- and zero-order enzyme kinetics. It is also of great interest that production of circulating $1,25(OH)_2D$ in the fetus is linked directly to circulating 25(OH)D [36].

Vitamin D metabolism is greatly altered during pregnancy, and pregnancy itself is the primary driver for these extraordinary circulating $1,25(OH)_2D_3$ levels. From our data, it is evident that production of $1,25(OH)_2D_3$ is really not under the control of the classic regulators of calcium, phosphorus, and PTH. The dramatic rise in maternal circulating $1,25(OH)_2D_3$ following conception is remarkable for many reasons: by 12 weeks of gestation, maternal circulating $1,25(OH)_2D_3$ levels are already triple those of a nonpregnant female [1]. From that point in gestation, $1,25(OH)_2D_3$ levels rise much higher and are driven by substrate—25(OH)D—availability (Fig. 2). This substrate dependence of $1,25(OH)_2D_3$ production is never observed in normal human physiology driven by classic calcium homeostasis.

Another remarkable factor in pregnant women is how they can attain supraphysiologic levels of $1,25(OH)_2D_3$, sometimes exceeding 700 pmol/L in our study, and yet never exhibit hypercalciuria or hypercalcemia [1]. These supraphysiological circulating levels of $1,25(OH)_2D_3$ during pregnancy are possibly of placental origin or from the renal 1-α-hydroxylase that would have to be uncoupled from feedback control and for reasons other than maintaining calcium homeostasis. The second scenario is most likely because women with nonfunctional renal 1-α-hydroxylase and normal placental function fail to increase circulating $1,25(OH)_2D_3$ during pregnancy [37]. The increased levels of $1,25(OH)_2D_3$ may be due to methylation of the catabolic *CHP24A1* placental gene [38]. Calcitonin may be a contributor to this process in that it rises during pregnancy [39], is known to stimulate the renal 1-α-hydroxylase gene independently of calcium levels [40, 41], and protects by opposing hypercalcemia [42]. Another possible stimulator of 1-α-hydroxylase during pregnancy is prolactin [43]. If prolactin were a major contributor, however, the effect should continue into lactation, which we do not see, and would be accompanied by elevated circulating $1,25(OH)_2D_3$ levels, which also are not seen [44]. Clearly, vitamin D metabolism during pregnancy is unique in human physiology; but what is its purpose?

What Constitutes Vitamin D Deficiency during Pregnancy?

What circulating level of 25(OH)D [9] does a pregnant woman require to be considered replete? The 2010 IOM report states that if one exhibits a circulating 25(OH)D level of 50 nmol/L, then that individual should be considered replete [8]. That document also stated that individuals consuming an adequate diet would receive enough vitamin D from that diet without consideration of race, latitude, or season and would not require a dietary supplement. Yet, there is a plethora of data from numerous studies throughout the world in the last decade that

suggests otherwise [9]. Fortunately, new clinical guidelines have recently been released by the Endocrine Society that provide serious guidance to the vitamin D deficiency problem [9].

Let us compare the two report recommendations for vitamin D with respect to pregnancy. As mentioned earlier, the IOM recommended a circulating level of 25(OH)D of 50 nmol/L, whereas the Endocrine Society recommends a level of more than 75 nmol/L [8, 9]. To achieve the 50 nmol/L, the IOM recommends 400–600 IU/day, which it states can be obtained through dietary means without supplementation [8]. In contrast, the Endocrine Society recommends an intake of 1,500–2,000 IU/day to achieve a circulating 25(OH)D level of more than 75 nmol/L [9]. How could these recommendations be so divergent? The IOM chose to use only RCT data limited to skeletal integrity, whereas the Endocrine Society chose to use a combination of peer-reviewed basic and clinical scientific publications [8, 9]. The reader will have to decide which report is best to adopt and use for patient guidance.

To examine the actual vitamin D deficiency rates during pregnancy, two recent publications provide some important insight. Even when applying the recent IOM normative 25(OH)D range of 50 nmol/L to the data [8], Hamilton et al. [45] and Johnson et al. [46] both provide shocking deficiency rates during pregnancy in a sunny climate. This work confirms an earlier study by Lee et al. [47] that documented high deficiency rates in mothers consuming prenatal vitamins. This deficiency problem is especially severe in minority populations. The IOM report claims that individuals obtain enough vitamin D from their diet to achieve the circulating 25(OH)D level minimum of 50 nmol/L. This statement is in direct conflict with two recent supplementation studies during pregnancy that failed to achieve this minimum circulating requirement for 25(OH)D [47, 48].

Finally, we have proposed to use mathematical models based on clinical data to dictate what would constitute vitamin D deficiency in the pregnant subject [1]. This is actually a simple task if one inspects Figs. 1, 2 and chooses the inflection point between first- and zero-order enzyme kinetics. These models give the level of at least 100 nmol/L circulating 25(OH)D to support maximal $1,25(OH)_2D$ production during pregnancy by overcoming "substrate limitation."

Consequences of Vitamin D Deficiency during Pregnancy

Calcium Homeostasis and Skeletal Integrity

At the top of any list concerning vitamin D deficiency, calcium homeostasis and skeletal integrity remain a top priority. What is really surprising is the relative dearth of information that exists between this topic and its effect on the pregnant woman and her fetus. There are no RCT data to guide us here, and thus, we must rely on observational data. A superb observational study by Yorifuji et al. [49] provides data to suggest that craniotabes in the newborn infant is the earliest sign of subclinical vitamin D deficiency during pregnancy. At 1 month of age, infants with craniotabes had significantly higher serum alkaline phosphatase, intact PTH, and lower circulating 25(OH)D levels than infants not exhibiting craniotabes. Several other recent observational studies have linked poor nutritional vitamin D status to abnormalities in both maternal and fetal skeletal markers and skeletal integrity itself [50–53]. It is also worth mentioning an animal model study that suggests pregnancy itself upregulates intestinal calcium absorption and skeletal mineralization independently of vitamin D, suggesting that vitamin D is not required for the skeletal adaptations during pregnancy [54]. However, this study was performed in mice, and its relevance to human physiology remains to be established. One additional observation is that in utero vitamin D levels may impart a positive impact on skeletal integrity later in life [55].

Data from our recent RCT tend to support the relative lack of effect of vitamin D on the calcium and skeletal homeostatic systems as implied in the animal model [1, 54]. As discussed earlier in this review, circulating $1,25(OH)_2D$ levels in the pregnant human elevate to supraphysiologic levels without any classic stimulus such as low serum calcium or increased PTH [1]. Further, in spite of these huge circulating $1,25(OH)_2D$ levels, serum and urinary calcium levels are normal [1]. The only observed changes in classic calcium homeostatic parameters we observed in our patients were due to normalizing circulating 25(OH)D levels, and they were (1) lowering PTH levels in vitamin D deficient African Americans and (2) a normalization of urinary calcium excretion [1].

Alterations in Immune Function

The control of immune function, both adaptive and innate by nutritional vitamin D status, is a very active area of investigation with regard to pregnancy. Liu et al. [56], using the mouse as an experimental animal for assessing vitamin D's role in the regulation of placental inflammation, determined that maternal and fetal vitamin D levels play a pivotal role in controlling placental inflammation. Does vitamin D play a similar role in human pregnancy? Current data suggest that it does. Walker et al. [36] have shown that cord blood vitamin D status in human participants controls the innate immune response. This study demonstrated that cord blood vitamin D deficiency, by its effect on toll-like receptor–induced antimicrobial production, altered in vitro monocyte responses [36]. The result of

this defect would be decreased barrier protection against invading pathogens. Actual observational data suggest that this is, in fact, the case.

Belderbos et al. [57] demonstrated that nutritional vitamin D deficiency in otherwise healthy neonates is associated with subsequent increased risk of respiratory syncytial viral bronchiolitis. Further, maternal vitamin D deficiency is associated with bacterial vaginosis, and this deficiency may contribute to the strong racial disparity in the prevalence of bacterial vaginosis [58, 59]. Similar mechanisms, with respect to vitamin D deficiency and innate immune function, likely contribute to periodontal disease during pregnancy [60]. Low circulating 25(OH)D levels also have been linked to the risk of respiratory infection, wheezing, and asthma [61–63] and have an apparent impact on the markers of severity of childhood asthma [64], possibly by altering T-regulatory cells [65]. Our recent RCT, using the intent-to-treat model, failed to demonstrate a relationship with vitamin D supplementation and infection [1] (Table 2). Finally, one of the most important aspects of vitamin D's proposed interactions with the adaptive immune system involves its potential to alter multiple sclerosis susceptibility of the infant later in life by improving nutritional vitamin D status during pregnancy [66–70].

Complications and Outcomes of Pregnancy

Complications of pregnancy include preeclampsia, gestational diabetes, and hypertension. Although these complications of pregnancy are well known and contribute to morbidity and mortality during pregnancy, their association with nutritional vitamin D status is a new area of investigation. Preeclampsia is a multisystem disorder that complicates 3–8 % of pregnancies in Western countries and constitutes a major source of morbidity and mortality worldwide [68, 69]. Overall, 10–15 % of maternal deaths are directly associated with preeclampsia and eclampsia.

Some epidemiological findings support the hypothesis of a genetic and immunological etiology. The risk of preeclampsia is two- to fivefold higher in pregnant women with a maternal history of this disorder. Depending on ethnicity, the incidence of preeclampsia ranges 3–7 % in healthy nulliparas and 1–3 % in multiparas. Other risk factors have been identified, including a medical history of chronic hypertension, kidney disease, diabetes, obesity, birthplace in Africa, age $\geq$35 years, pregnancy characteristics such as twin or molar pregnancy, previous preeclampsia, and fetal congenital abnormality [71, 72].

Preeclampsia may be life-threatening for both mother and child, increasing both fetal and maternal morbidity and mortality and often leading to preterm delivery of the fetus due to worsening preeclampsia [69]. In the mother, preeclampsia may cause premature cardiovascular disease, such as chronic hypertension, ischemic heart disease, and stroke, later in life [73]. Children born after preeclamptic pregnancies and who are relatively small at birth have an increased risk of stroke, coronary heart disease, and metabolic syndrome in adult life [74–76].

While the sole curative treatment of preeclampsia is delivery, management of the preeclamptic woman must continuously balance the risk to benefit ratio of induced preterm delivery and maternal–fetal complications. Further, no drug intervention is known to prevent preeclampsia. There are intriguing possibilities that vitamin D is integral in maintaining normal placental integrity and function [77]. Bodnar et al. [78] first described the relationship between poor vitamin D status and risk of preeclampsia. Additional observational studies have strengthened this observation in the past year. Baker et al. [79], using a nested case–control study, found that maternal mid-gestation vitamin D deficiency was associated with increased risk of severe preeclampsia. Robinson et al. [80], utilizing a case–control investigation with gestation-matched contemporaneous control participants, determined that circulating 25(OH)D levels were significantly decreased in early-onset severe preeclamptic individuals. This group further demonstrated that 25(OH)D levels are lower among SGA patients in early-onset severe preeclampsia than those infants without growth retardation [81]. It was concluded from this study that vitamin D status may impact fetal growth through placental mechanisms. Data from our RCT do not suggest a positive or negative effect between vitamin D and birth weight (Table 2) [1]. Finally, Wei et al. [82] recently published a longitudinal study that clearly demonstrated an inverse relationship between circulating 25(OH)D during pregnancy and preeclampsia.

In contrast to prior studies, Bodnar et al. [83] published an observational study linking both low and high nutritional vitamin D status to an increased risk of SGA births in only white women. Data from our RCT do not support this premise (Table 3). In fact, data from our study do not demonstrate any risk of SGA associated with vitamin D supplementation in our Caucasian population at the higher levels of vitamin D (Table 3). It is still possible that lower levels of vitamin D may contribute to SGA births, but this cannot be ascertained from our data. As for African Americans and Hispanics, our data are not clear and offer no trends. What is clear is that an RCT would be necessary for this relationship to be assessed, the cost and time of which would be enormous. Along the spectrum of hypertensive disorders of pregnancy, additional evidence of vitamin D's role during pregnancy comes from an observational study by Ringrose et al. [84], who found a high prevalence of vitamin D deficiency in pregnant women in

Table 2 Pregnancy characteristics and outcomes by vitamin D supplementation group controlling for race

Characteristic	400 IU ($n = 111$)	2,000 IU ($n = 122$)	4,000 IU ($n = 117$)	p	p, 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)	61.2 ± 27.1	57.6 ± 22.4	59.8 ± 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 (g) at delivery (mean ± SD)	3,222 ± 675	3,360 ± 585	3,285 ± 598	0.23	0.2
Mode of delivery[a], *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 C/S	24 (25.3 %)	22 (20.6) %	15 (14.3 %)	0.15	0.046
Previous PTB, *n* (%)	20 (18.0 %)	32 (26.2 %)	23 (19.7 %)	0.27	0.9
PTB < 37 weeks, *n* (%)	9 (8.1 %)	5 (4.1 %)	7 (6.0 %)	0.44	0.5
PTL < 37 weeks in this pregnancy, *n* (%)	16 (14.4 %)	22 (18.0 %)	14 (12.0 %)	0.41	0.4
PTL/PTB < 37 weeks, *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, *n* (%)	47 (42.3 %)	60 (49.2 %)	44 (37.6 %)	0.19	0.4
Any					
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
Comorbidity (PTB), *n* (%) (infection, PTB, gestational diabetes, preeclampsia/hypertension/HELLP)	63 (56.8 %)	67 (54.9 %)	53 (45.3 %)	0.17	0.06
Comorbidity (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

[a] Mode of delivery was categorized a priori as either vaginal (defined as spontaneous or assisted vaginal delivery, which included use of forceps or vacuum extraction) or cesarean section (C/S; further subdivided as cesarean following labor, cesarean without labor, and repeat elective cesarean). Primary cesarean section included women who had undergone a cesarean section with or without labor for either a maternal or a fetal indication and did not include women who underwent a repeat, elective cesarean section

PTB preterm birth, *PTL* preterm labor

From Hollis et al. [1]

Canada and that this deficiency was independently linked to hypertension in these women that may be regulated by flow-mediated dilation [85].

A recent observational study by Lau et al. [86] has provided us with a link between low vitamin D levels and gestational diabetes mellitus. Lau et al. [86] found that circulating 25(OH)D levels were inversely associated with fasting, 2 h blood glucose levels during glucose tolerance testing and glycated hemoglobin levels (HbA_{1c}). Multivariate analysis identified 25(OH)D and glucose levels as independent predictors of HbA_{1c}. Thus, low 25(OH)D levels were associated with poor glycemic control during pregnancy. A recent study by Parlea et al. [87] suggests that vitamin D may influence glucose tolerance during pregnancy and supports studies of vitamin D as a potential intervention to prevent gestational diabetes. Also, recent observational studies have linked vitamin D deficiency to an increase in primary cesarean section [88] and premature delivery [89].

The risk of adverse events during pregnancy was not increased in the higher-dose vitamin D supplementation groups in our recent clinical trial [1]. We evaluated women

Table 3 Number and percentage of small-for-gestational-age (SGA) infants by vitamin D treatment group

Racial group	Number of subjects	400 IU/day	2,000 IU/day	4,000 IU/day
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 %)

Data derived from Hollis et al. [1]

monthly throughout their pregnancies starting at 12 weeks' gestation. To be eligible for the study, women had to be in good general health at the time of enrollment without a history of hypertension or diabetes. When reviewing combined or cumulative comorbidities of pregnancy, the data from our RCT strongly suggest that vitamin D supplementation during pregnancy can significantly decrease complications of pregnancy including primary cesarean section ($p = 0.046$), hypertensive disorders of pregnancy ($p = 0.05$), and comorbidities of pregnancy ($p = 0.03$) [1] (Table 2).

Lack of in utero vitamin D has also been associated with abnormal brain development in experimental animals [90]. In humans, vitamin D during pregnancy has been associated with risk of schizophrenia at both low and higher levels of circulating 25(OH)D [91]. In this study, the highest quintile of circulating 25(OH)D was 51 nmol/L, and to suggest that these levels would result in increased schizophrenia rates would be puzzling indeed since levels in native Africans are at least three times this level and have been for hundreds of thousands of years [19]. In the latest evidence, with respect to offspring and neurocognitive development, Whitehouse et al. [92] demonstrated that maternal vitamin D insufficiency during pregnancy is significantly associated with offspring language impairment.

Summary

Meaningful research with regard to vitamin D supplementation in pregnant women has been hampered for decades by misconceived dietary recommendations and fear of toxicity, which have been refuted [93]. Current vitamin D intake recommendations during pregnancy range from 400–600 IU/day from the IOM report [8] to 1,500–2,000 IU/day from the Endocrine Society report [9]. The American College of Obstetricians and Gynecologists (ACOG) has chosen to follow the IOM report [94]: the ACOG document states that, "Vitamin D screening and supplementation during pregnancy is not required 'unless' women live in cold climates, reside in northern latitudes, wear sunscreen and protective clothing, are ethnic minorities, or are vegetarian." This "unless" group basically defines the entire North American and European populations. Many recent observational studies now exist that present strong evidence of the positive effects that vitamin D can provide by improving birth outcomes [78–88]. When we conceived our vitamin D supplementation pregnancy RCT in 2001, we simply asked the question, how much vitamin D would be required to increase circulating 25(OH)D levels to achieve levels that mimic those obtained due to significant solar exposure and provide levels that have been inherent in humans for most of our evolution [19, 95]? We anticipated that amount to be several thousand IUs per day. After convincing the FDA that our proposed study was designed to establish efficacy and effectiveness while minimizing risk, we received investigation drug approval, and the study was undertaken and completed. However, one of the most puzzling developments was the omission of our RCT pregnancy study from the most recent Cochrane review on vitamin D supplementation for women during pregnancy [30]. Without inclusion of our RCT, which included a control group appropriate for a large section of the world today, the world literature and the recommendations for vitamin D supplementation continue to remain at odds.

How did our assumption about natural historical human levels and supplementation turn out? The results actually are quite remarkable and provide strong evidence of the positive effects of vitamin D on birth outcomes without any hint of adverse effects. The daily intake of vitamin D to accomplish these results was 4,000 IU. Our 4,000 IU/day group achieved 111.0 nmol/L circulating 25(OH)D (Table 1), while values in traditionally living populations in East Africa are known to be 115 nmol/L [19] but significantly less than is observed in native African women who are pregnant (M. Luxwolda, personal communication). We believe that this is the circulating level of 25(OH)D we should aspire to attain, not a level based on geometric means from populations that live in sun-restricted environments, covered with clothing, and told to avoid the sun at all costs to the point of a skin mutation that maximizes limited solar irradiation [10, 16]. We believe that one should assimilate solid historical data with data generated from modern techniques [1, 19] and not be wedded to ideas that came from intuition that has simply carried forth for decades [7]. Our rigorous and well-designed RCT accessing vitamin D supplementation during pregnancy, overseen by both the National Institutes of Health and the FDA, provides clear evidence of vitamin D's role in nonskeletal health outcomes in pregnancy. Basically, it is our perspective with regard to vitamin D and pregnancy that our genome has developed to >60 ng 25(OH)D/mL (150 nmol/L) since the beginning of humankind, but we are so arrogant as to believe that levels in sun-starved humans are

"normal." Worse, we think these levels in which our genome evolved are actually harmful in some way, so we have endless data safety and monitoring boards to evaluate 25(OH)D levels that are totally natural but now in some way doing us harm. The next step in this saga will be to evaluate the evidence based on historical precedence and accumulating data that will en masse supersede previously held dogma.

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JBMR

Vitamin D Supplementation During Pregnancy: Double-Blind, Randomized Clinical Trial of Safety and Effectiveness

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ABSTRACT

The need, safety, and effectiveness of vitamin D supplementation during pregnancy remain controversial. In this randomized, controlled trial, women with a singleton pregnancy at 12 to 16 weeks' gestation received 400, 2000, or 4000 IU of vitamin D_3 per day until delivery. The primary outcome was maternal/neonatal circulating 25-hydroxyvitamin D [25(OH)D] concentration at delivery, with secondary outcomes of a 25(OH)D concentration of 80 nmol/L or greater achieved and the 25(OH)D concentration required to achieve maximal 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] production. Of the 494 women enrolled, 350 women continued until delivery: Mean 25(OH)D concentrations by group at delivery and 1 month before delivery were significantly different ($p < 0.0001$), and the percent who achieved sufficiency was significantly different by group, greatest in 4000-IU group ($p < 0.0001$). The relative risk (RR) for achieving a concentration of 80 nmol/L or greater within 1 month of delivery was significantly different between the 2000- and the 400-IU groups (RR = 1.52, 95% CI 1.24–1.86), the 4000- and the 400-IU groups (RR = 1.60, 95% CI 1.32–1.95) but not between the 4000- and. 2000-IU groups (RR = 1.06, 95% CI 0.93–1.19). Circulating 25(OH)D had a direct influence on circulating $1,25(OH)_2D_3$ concentrations throughout pregnancy ($p < 0.0001$), with maximal production of $1,25(OH)_2D_3$ in *all* strata in the 4000-IU group. There were no differences between groups on any safety measure. Not a single adverse event was attributed to vitamin D supplementation or circulating 25(OH)D levels. It is concluded that vitamin D supplementation of 4000 IU/d for pregnant women is safe and most effective in achieving sufficiency in all women and their neonates regardless of race, whereas the current estimated average requirement is comparatively ineffective at achieving adequate circulating 25(OH)D concentrations, especially in African Americans. © 2011 American Society for Bone and Mineral Research.

KEY WORDS: VITAMIN D; CHOLECALCIFEROL; PREGNANCY; NEONATE

Introduction

The function of vitamin D during pregnancy for both mother and fetus remains largely undefined. Vitamin D is known to be involved in skeletal homeostasis during pregnancy, as evidenced by a recent publication dealing with craniotabes in the newborn, and severe vitamin D deficiency may lead to neonatal seizures in neonates with profound hypocalcemia.[1–5] The function of vitamin D during this sensitive period, however, also may have potential effects on other systems, including immune,[6–10] pancreatic,[11–13] musculoskeletal,[14–17] and cardiovascular function,[18–20] as well as neural development.[21–24] Recent publications suggest relationships between maternal vitamin D status and adverse pregnancy outcomes such as preeclampsia and cesarean section.[25–28]

A Cochrane Review published in 2000 highlighted the relative dearth of data dealing with vitamin D supplementation during human pregnancy.[29] This review listed seven studies on the topic,[30–36] of which four reported clinical outcomes.[30–32,36] From these limited data, the Cochrane Review concluded that there was insufficient evidence to evaluate the effects of vitamin D supplementation during pregnancy.[29] Since that time, few studies have addressed this issue.[37–39]

In 2004, we initiated a National Institute of Child Health and Human Development (NICHD)–sponsored 6-year randomized, double-blind, placebo-controlld trial of vitamin D supplementa-

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tion during pregnancy to assess safety and pregnancy outcomes with an approved Investigational Drug Application from the US Food and Drug Administration (FDA; No. 66,346). We hypothesized that 4000 IU/d of vitamin D_3 would be more efficacious and effective than the standard dosing regimen of 400 IU/d and the 2000 IU/d (the former upper limit for vitamin D) dosing regimen in achieving a total circulating 25-hydroxyvitamin D [25(OH)D] level of at least 80 nmoL/L (32 ng/mL) in pregnant women regardless of race throughout pregnancy and at the time of delivery without causing any safety concerns. This minimal value of 80 nmol/L was based on years of research with regard to circulating 25(OH)D levels suppressing secondary hyperthyroidism and having optimal intestinal calcium absorption and bone mineral density.[41] These results are presented here.

Methods

Study design

This study was a single-center, randomized, controlled, double-blind study of vitamin D supplementation stratified by race (FDA IND No. 66,346; ClinicalTrials.gov No. NCT00292591). Women at fewer than 16 weeks' gestation with a singleton pregnancy were eligible for participation in the study.

Study participants and setting

This study was approved by Medical University of South Carolina's (MUSC's) Institutional Review Board for Human Research (HR No. 10725) and was conducted from January 4, 2004, through July 31, 2009, at MUSC (Charleston, SC, USA). The inclusion criteria for the subjects included the following: (1) maternal age of 16 years or greater at the time of consent, (2) confirmed singleton pregnancy of fewer than 16 completed weeks of gestation at the time of consent, (3) planned to receive ongoing prenatal care in the Charleston, SC, area, and (4) the ability to provide written informed consent at the first visit. If a woman received her obstetrical care at a facility separate from MUSC, then she came to MUSC's Clinical and Translational Research Center (CTRC) outpatient research facility for each of the study visits. Women were consented at their first prenatal visit, at which time baseline 25(OH)D levels were measured. Irrespective of gestational age at enrollment, subjects began vitamin D supplementation between the start of the twelfth and the start of the sixteenth weeks of gestation (12 0/7th and 16 0/7th weeks), as defined by their last menstrual period.

Exclusion criteria

Women with a pregnancy at greater than 16 weeks of gestation as calculated by their last menstrual period were not eligible to participate. Pregnant women with preexisting calcium or parathyroid conditions or who required chronic diuretic or cardiac medication therapy, including calcium channel blockers, or who suffered chronic hypertension were not eligible for enrollment in the study. Pregnant women with active thyroid disease (eg, Graves disease, Hashimoto disease, or thyroiditis) also were excluded, but mothers on thyroid supplement with normal serologic parameters could participate in the study if they were without any other endocrine dysfunction.

Study protocol

Gestational age at enrollment

Subjects could be consented and enrolled into the study before the initiation of vitamin D supplementation at 12 to 16 weeks of gestation. Gestational age was based on last menstrual period. If a woman was unsure of her gestational age, the obstetrical estimate at the time of the visit was used. If, at the 20-week fetal ultrasound it was determined by the obstetrician that the gestational age was incorrect, the revised gestational age was used and the discrepancy noted.

Initial study visit

Baseline blood and urine samples were obtained following subject consent at the initial visit, but the earliest time of randomization following measurement of baseline total circulating 25(OH)D level was 12 weeks' gestation, with the target upper limit of gestation of 16 weeks. Irrespective of enrollment gestational age, vitamin D supplementation did not begin before the twelfth week of gestation (12 and 0/7th weeks).

Subsequent study visits

Subjects were followed with monthly study visits, which continued until delivery. The visits coincided with routine obstetrical visits or were performed in conjunction with those visits if the obstetrical care was provided outside MUSC. The subjects also were seen at the GCRC/CTRC for a study visit at 16 weeks of gestation and with their infant at 2 weeks' postpartum.

Completion of questionnaires

Following their written informed consent, mothers completed questionnaires regarding sociodemographic information, baseline health status, and medical history at the first visit. At the second visit, the Block Food Frequency Questionnaire (FFQ) was completed to ascertain generalized eating pattern, with specific calculation of calcium and vitamin D intake (Block, Berkeley, CA, USA).[42–47] Each completed FFQ form was sent to the processing center (Berkeley, CA, USA), and these data were reviewed later for accuracy by a registered dietician who was blinded to subject treatment group assignment. Total caloric, vitamin D, and calcium intakes were recorded for each subject.

An interim maternal health history questionnaire also was completed at each visit with the assistance of the study coordinator to ascertain adverse events, discussing types and frequencies of acute illnesses such as respiratory, gastrointestinal, and other viral and/or bacterial illnesses. A review of medications and doctor's visits was obtained at that time.

After delivery, the newborn record of each infant was reviewed for mode of delivery and level of neonatal care required (normal newborn nursery or level 2 or level 3 intensive care). Birth weight (g) and gestational age also were recorded.

Blood and urine samples

Maternal blood and urine samples were collected at each visit. Cord blood was obtained at delivery. If the cord blood sample

could not be obtained, a neonatal blood sample was drawn within 2 weeks of delivery.

Intervention

Multivitamin and vitamin D supplementation

Pregnant women who presented for prenatal care at 16 or fewer weeks of gestation were randomized into one of three treatment regimens of vitamin D_3 after establishing their baseline serum 25(OH)D level. All patients received a total of two pills daily: a standard prenatal multivitamin vitamin containing 400 IU of vitamin D and an additional vitamin D_3 supplement of 0 IU (placebo), 1600 IU, or 3600 IU of vitamin D_3 for a total of 400 IU, 2000 IU, and 4000 IU of vitamin D supplementation, respectively.

In order to obtain Institutional Review Board approval for the study, the following safety measure was put into place: Baseline total circulating 25(OH)D levels were measured, and women with levels of 100 nmol/L (40 ng/mL) or less were eligible for randomization into one of the three arms (400, 2000, or 4000 IU/d of vitamin D_3) with further substratification by race within each treatment group. Women with baseline 25(OH)D levels greater than100 to 150 nmol/L (>40 to 60 ng/mL, levels considered to be in the normal range at the time of study implementation) were randomized into one of two treatment groups (400 or 2000 IU/d of vitamin D_3), whereas women with a baseline 25(OH)D level greater than 150 nmol/L (>60 ng/mL) were given 400 IU/d of vitamin D_3. The doses of vitamin D used in our study were selected based on current recommendations (400 IU/d), the upper safe intake level established in 1997 (2000 IU/d),[40] and the amount we calculated to be required to achieve nutritional vitamin D sufficiency (4000 IU/d).[48].

Adherence to medication regimen

Adherence to the prescribed vitamin D supplementation regimen of one prenatal vitamin and the vitamin D supplement was measured by maternal self-report and pill counts at each follow-up visit.[49] The number of vitamin D pills returned was divided by the expected number of pills that would have been taken between study visits to generate a percentage that served as a measure of adherence of medication regimen between study visits. The adherence measures were used to generate an average adherence for each subject.[49] If a woman missed one prenatal visit, her next month supply of vitamins was either mailed to her or dropped off at her residence. In such cases, medication adherence was based on the pill count from the date of the last visit to the current prenatal visit over the expected number of pills taken. If a woman had more than two missed visits or if she failed to take at least 50% of the prescribed vitamin D pills, she was exited from the study.

Randomization

Our study used stratified blocked randomization to balance by ethnicity and also to balance by enrollment (as a cautionary measure against a potential temporal or seasonal bias). A randomization scheme was developed separately for each of the three ethnic groups (ie, the strata). Within each stratum, the treatments were assigned within blocks. Because there were three treatment groups, the block size had to be divisible by 3; the data team selected a block size of six, which was unknown to the investigators or the pharmacists. In this way, at the end of each block (ie, enrollment of six subjects), each ethnic group was balanced in the number randomly assigned to the 400-, 2000-, and 4000-IU treatment groups.

Materials

Source of vitamin D

Vitamin D tablets were manufactured by Tishcon Corporation (Westbury, NY, USA), a Good-Manufacturing-Practice (GMP) facility. The cholecalciferol contained in the vitamin D tablet was supplied to Tishcon Corporation by Hoffman-La Roche, Ltd. (Basel, Switzerland). The tablet vitamin D concentration was verified by the company every 6 months and by an independent laboratory chosen by the investigators (Heartland Assays, Ames, IO, USA) using high-performance liquid chromatography with UV detector (HPLC-UV) to ensure that the tablets met label claims throughout the study; these results were reported to the Investigational Drugs Department at MUSC. Tablets were maintained in MUSC's Research Pharmacy until the time that they were dispensed to each enrolled subject.

Source of prenatal vitamins

Prenatal vitamins prescribed at the time of each subject's enrollment were manufactured by Myadec Multivitamin-Multimineral Supplement (distributed by Pfizer Consumer Healthcare, Morris Plains, NJ, USA) with 400 IU of vitamin D_3 per tablet. Mothers who were unable to swallow a prenatal vitamin were given Flintstones Complete chewable vitamin (Bayer Healthcare, Morristown, NJ, USA), which provided 400 IU of vitamin D_3 per tablet.

Measures

Maternal sociodemographic measures included maternal age at time of enrollment, her self-defined race, insurance status, educational status, and occupation and employment outside of the home.

Pregnancy health status and labor and delivery characteristics and complications

Characteristics of each mother's health status and complications during pregnancy, labor, and delivery were recorded and reviewed by an obstetrician (DDJ, blinded to treatment). If the mother required hospitalization, a copy of the hospital record was obtained after the mother had signed a release of medical information form. Any acute illnesses, hospitalizations, or development of pregnancy-related conditions that were not preexisting also were recorded. The Data Monitoring and Safety Committee (DSMC) was notified of all such events.

Anthropomorphic measurements

Prepregnancy height and weight of each mother were recorded at the first outpatient visit to determine BMI (weight [kg]/height^2

[m^2]). During subsequent visits, only the subject's weight was recorded. Birth weight (g) was recorded for each infant.

Laboratory measurements

Maternal and cord blood/neonatal vitamin D and metabolite assays

Circulating vitamin D_2 and D_3 were measured in serum using direct ultraviolet detection preceded by organic extraction and high-performance liquid chromatography, as described previously.[50] This assay has a coefficient of variation of 10% or less and a 5 nmol/L vitamin D detection limit. There is no normal established circulating range of vitamins D_2 or D_3 in human subjects.

A rapid, direct RIA developed in the Hollis laboratory and manufactured by Diasorin Corporation (Stillwater, MN, USA) was used to measure total circulating 25(OH)D concentration in serum samples.[51] This RIA is an FDA-cleared device and, in fact, is the FDA predicate device for the measurement of circulating 25(OH)D in humans.

Based on clinical laboratory classifications,[52,53] a priori, deficiency was defined as a total circulating 25(OH)D level of less than 50 nmol/L (20 ng/mL), insufficiency as 50 nmol/L or greater to less than 80 nmol/L ($\geq$20 to $<$32 ng/mL), and sufficiency as 80 nmol/L or greater ($\geq$32 ng/mL).[41,53–55] The inter- and intraassay coefficients of variation are 10% or less.

An RIA manufactured by Diasorin Corporation and developed in the Hollis laboratory was used to measure total circulating 1,25-dihydroxyvitamine D_3 [1,25$(OH)_2D_3$] concentration.[56] This assay uses an ^{125}I-labeled tracer, and samples are processed using acetonitrile followed by solid-phase extraction and quantitation. This RIA is an FDA-cleared device. The normal circulating level of 1,25$(OH)_2D_3$ is 48 to 120 pmol/L (20 to 50 pg/mL). The inter- and intraassay coefficients of variation are 15% or less.

Maternal and cord blood/neonatal circulating intact parathyroid hormone (PTH) concentrations

Intact PTH (iPTH) was measured by immunoradiometric assay (IRMA) that uses two different polyclonal antibodies (Diasorin). The first antibody, specific for PTH(39–84), is bound to a solid-phase bead. The second antibody is specific for PTH(1–34) and is labeled with ^{125}I. The adult normal range for iPTH in our laboratory is 1.3 to 5.4 pmol/L. Higher vitamin D levels are associated with lower iPTH levels because iPTH declines as vitamin D status improves.[57]

Maternal baseline and follow-up serum calcium, creatinine, and phosphorus studies

Maternal serum total calcium, creatinine, and inorganic phosphorus levels were measured by MUSC's Clinical Chemistry Laboratory using standard methodology and laboratory normative data. Results were reported to the clinical principal investigator (PI; CLW) and downloaded to the research database from the clinical chemistry registry. All results were reviewed by the clinical principal investigator of the study on a weekly basis for any abnormal values and reported to the DSMC.

Circulating vitamin D–binding protein (VDBP)

VDBP was measured using a commercial ELISA purchased from R&D Systems (Minneapolis, MN, USA). Circulating VDBP levels in normal individuals using this ELISA are stated by the manufacturer to be 3.93 ± 1.62 μmol/L.

Maternal urinary calcium/creatinine ratio

A nonfasting urine sample was obtained from the mother at each obstetrical visit and was sent to the Clinical Chemistry Laboratory at MUSC for urinary calcium (Ca) and creatinine (Cr) measurements and derivation of the urinary Ca:Cr (mg/dL) ratio (converted to mmol/L/mmol/L). (To convert mg/dL of calcium to mmol/L, multiply the value by 0.25. To convert mg/dL of creatinine to mmol/L, multiply by 0.088.)

Safety measures throughout the study

All study subjects were monitored for hypervitaminosis D. The circulating 25(OH)D level of 225 nmol/L (90 ng/mL) was used to define hypervitaminosis D, as required by the FDA and our IRB. This conservative maternal level was arbitrarily chosen to ensure the safety of all study patients, particularly those assigned to the 4000 IU of vitamin D_3 per day regimen.[58] Subsequent vitamin D supplementation trials have demonstrated that circulating levels of 25(OH)D exceeding 300 nmol/L (120 ng/mL) do not cause hypercalciuria, the first indicator of hypervitaminosis D.[48] Even in women who are vitamin D deficient, urinary calcium excretion increases during pregnancy secondary to increased glomerular filtration rate.[59] Given this, urinary calcium/creatinine ratio was used and is the most sensitive early indicator of hypervitaminosis D. Operationally, we defined a priori *caution* limits for hypervitaminosis D as a nonfasting urinary calcium/creatinine ratio of 0.8 mg/mg or 2.27 mmol/mmol or greater.

Whenever any patient exceeded the caution limit or had an abnormal clinical chemistry value, a specific case study by the Data Safety and Monitoring Committee (DSMC) was to be initiated to examine the contribution of confounding factors (eg, diet, sunlight exposure, etc.). Operationally, vitamin D_3 supplementation stopped if the urinary calcium/creatinine ratio exceeded 1.0 (mg/dL/mg/dL) *or* if the circulating 25(OH)D level exceeded 225 nmol/L (90 ng/mL).

Statistical methods

Sample size and power considerations

To detect a statistically significant increase in 25(OH)D by 10 ng/mL between any two groups, it was calculated to require a minimum of 32 patients per group at 90% power, $\alpha = 0.05$, two-tailed test for the primary analysis. This calculation assumed that the standard deviation of 25(OH)D measurements at a single time point was approximately 10, that there would be a low correlation ($r = 0.25$) between the baseline and final measurements, and that a substantial proportion (up to 50%) of participants may be lost to follow-up owing to moving, termination of care, or discontinuation of participation. Because the primary outcome—maternal and neonatal vitamin D status at or around the time of delivery—a prerequisite for inclusion in the final analysis was that the mother had to have had a live birth

and had to have subject participated until the day of delivery. Lastly, since one of the secondary goals of this study was to explore vitamin D differences by ethnicity, the three supplemented groups (400, 2000, and 4,000 IU/d) were balanced by ethnicity (equal numbers of whites, blacks, and Hispanic).

Statistical analysis

The main variables of interest were: (1) differences in mean maternal and infant total circulating 25(OH)D levels at the time of delivery between supplement groups (ANOVA), and (2) differences between supplement groups in the proportion of women achieving a 25(OH)D level of 80 nmol/L or greater within 1 month and at the time of delivery (chi-square). Secondary analyses employed chi-square for categorical variables; ANOVA or Student's *t* test, as appropriate, for normally distributed variables (with the Bonferonni option for pairwise analysis in ANOVA); and paired Student's *t* test for within-group changes from baseline to delivery. Multiple regression was used to assess the association between final vitamin D concentrations and baseline values, ethnicity, dose group, and the dose × race interaction. Stratified analysis was used to more fully explore any evidence of interaction. Variables that were not normally distributed were analyzed with Wilcoxon-Mann-Whitney test. The association between vitamin D [25(OH)D and $1,25(OH)_2D_3$] and urinary calcium/creatinine ratio was explored with a combination of exponential and linear models. Data were analyzed with SAS 9.22[60] (SAS Institute, Cary, NC, USA) and SigmaPlot software (Systat Software, Inc., San Jose, CA, USA).

The analysis was conducted as an intention-to-treat (ITT) study.[61] The ITT approach (effectiveness) compares the outcomes between supplement groups, as assigned, and makes no assumption regarding whether or not subjects were adherent to the dosing regimen. (Data on adherent subjects will be made available to any investigator on request following publication.) The ITT design was used as a measure of the effectiveness of increasing vitamin D levels via oral dosing. This approach presents a conservative finding of potential benefits that could be shown from a population- or public health–based intervention. (Adherence efficacy and outcome data with detailed pharmacokinetics will be presented in a separate article.)

Results

Study population

Figure 1 shows the enrollment, allocation, and follow-up of the women who participated in the trial. A total of 516 women were interviewed, and 502 consented to participate in this study and were randomly assigned to a treatment group: 166 were assigned to group 1 (400-IU group), 167 were assigned to group 2 (2000-IU group), and 169 were assigned to group 3 (4000-IU group). Of the 502 women consented, there were 23 women with a valid initial 25(OH)D greater than 100 nmol/L (40 ng/mL) who were not eligible for enrollment in the 4000-IU group: 2 black, 6 Hispanic, and 15 white women. Of those, 12 were enrolled into the 400-IU group, 10 were enrolled into the 2000-IU group, and 1 was enrolled in the 4000-IU group [the latter being a protocol deviation early in the study where one woman with a baseline 25(OH)D level of 41 ng/mL was randomized to the 4000-IU group]. Seventeen continued until delivery: 1 black, 6 Hispanic, and 10 white women; 10 were in the 400-IU group,

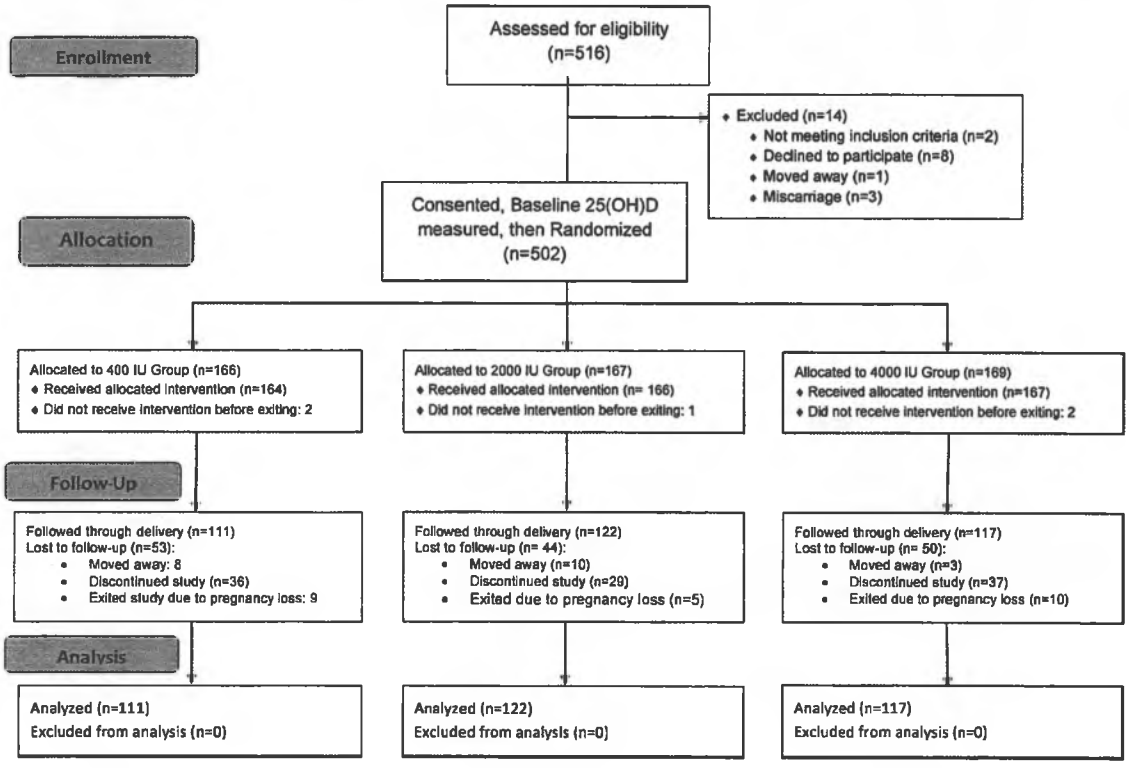


Fig. 1. Flow diagram of pregnancy study. IU = international units.

6 were in the 2000-IU group, and 1 was in the 4000-IU group. Finally, there was one white woman whose baseline 25(OH)D level was 172.5 nmoL/L (69 ng/mL) who was placed into the 400-IU group. After allocation into treatment groups, there were no statistically significant differences among the groups with regard to lost to follow-up, dropouts, or pregnancy losses.

The sociodemographic characteristics of the active cohort are found in Table 1. Baseline characteristics were similar between the groups on the basis of race/ethnicity, maternal age, gestational age at enrollment, educational and employment status, health rating, planned pregnancy, BMI, and season at study entry. There was a trend toward differences between the groups on the basis of maternal gravidity and parity and insurance status. A total of 62 women (12.4%) were taking a prenatal vitamin at the time of randomization. Of the 502 women who were randomized to treatment, 350 women continued in the study until delivery and had outcome data available for analysis: 98 black, 137 Hispanic, and 115 white women evenly distributed into the three treatment groups with 111 controls, 122 in 2000-IU and 117 in 4000-IU groups. There were no differences in baseline vitamin D status among treatment groups.

A comparison of women who completed the study and those who electively discontinued the study is found in Table 2. Women who had a pregnancy loss or who moved away were excluded from the analysis. Individuals who exited the study did not differ by treatment group. Women who electively exited the study were more likely to be black than Hispanic or white.

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

Maternal characteristic	400-IU group ($n = 111$)	2000-IU group ($n = 122$)	4000-IU group ($n = 117$)	p Value
Race/ethnicity,[a] n (%)				0.9
Black	28 (25.2)	37 (30.3)	33 (28.2)	
Hispanic	45 (40.5)	48 (39.3)	44 (37.6)	
White	38 (34.2)	37 (30.3)	40 (34.2)	
Maternal age (years), mean ± SD	27.0 ± 5.6	27.4 ± 5.7	26.6 ± 5.4	0.6
Range	18–41	17–41	17–44	
Gestation at enrollment (weeks), mean ± SD	12.5 ± 1.9	12.6 ± 1.6	12.4 ± 2.0	0.8
Range	7.1–18.4	8.4–17.6	6.4–21.4	
Maternal gravidity, median	2	2	2	0.08
Range	1–8	1–7	1–9	
Maternal parity, median	2	2	1	0.052
Range	0–5	0–7	0–9	
Education, n (%)				0.4
<HS education	18 (17.3)	23 (19.7)	13 (11.6)	
HS graduate	17 (16.4)	24 (20.5)	22 (19.6)	
College or more	69 (66.4)	70 (59.8)	77 (68.8)	
Employed at study entrance, n (%)	61 (55.0)	67 (54.9)	65 (55.6)	0.9
Insurance, n (%)				0.07
Medicaid/none	62 (55.9)	85 (69.7)	69 (59.0)	
Commercial	49 (44.1)	37 (30.3)	48 (41.0)	
Subjective health rating scale, Median[b]	9	10	10	0.4
Range	5–10	5–10	1–10	
Planned pregnancy, n (%)	59 (54.6)	61 (50.4)	59 (50.4)	0.8
BMI, n (%)[c]				0.6
≤30	78 (70.3)	87 (71.3)	89 (76.1)	
>30	33 (29.7)	35 (28.7)	28 (23.9)	
Season at study entry, n (%)				0.9
April–September	54 (48.7)	60 (49.2)	56 (47.9)	
October–March	57 (51.4)	62 (50.8)	61 (52.1)	
Vitamin D intake in IU,[d] mean ± SD	181.6 ± 108.4	195.8 ± 135.0	204.2 ± 148.2	0.6
Range	21.4–470.6	8.2–693.8	5.3–737.3	
Calcium intake, mg/d, Mean ± SD	1063.6 ± 539.6	993.9 ± 514.0	1073.6 ± 491.9	0.6
Range	252.9–2888.1	285.4–2754.1	275.6–2925.9	
kcal Intake, mean ± SD	2148.3 ± 778.6	2059.4 ± 803	2212.9 ± 920.8	0.5
Range	977.3–4668.2	993.4–4793.4	929.3–5516	

[a]Race/ethnicity as defined by mother.
[b]Self-reported maternal health status rating from 1 (poor) to 10 (excellent).
[c]BMI = prepregnancy body mass index.
[d]International units (IU): dietary intake calculated from the Block 1998 Food Frequency Questionnaire;[42,43] amount did not include prenatal vitamin intake.

Table 2. Subjects Who Completed the Study Compared With Subjects Exited Before Delivery[a]

Maternal characteristic	Delivered ($n = 350$)	Exited ($n = 129$)	p Value
Treatment group, *n* (%)			0.5
400 IU	111 (74.0)	39 (26.0)	
2000 IU	122 (79.7)	31 (20.3)	
4000 IU	117 (75.5)	38 (24.5)	
Ethnicity,[b] *n* (%)			0.003
Black	98 (66.7)	49 (33.3)	
Hispanic	137 (81.1)	32 (18.9)	
White	115 (81.0)	27 (19.0)	
Maternal age (years), mean ± SD	27.0 ± 5.6	25.5 ± 5.1	0.01
Range	17–44	18–42	
Gestational age at enrollment (weeks), mean ± SD	12.5 ± 1.8	12.1 ± 2.1	0.053
Range[c]	6.3–21.4	6.1–17.7	
Maternal gravidity, median (range)	2.0 (0–9)	3.0 (1–10)	<0.0001
Education, *n* (%)			0.01
<HS education	54 (73.0)	20 (27.0)	
HS graduate	63 (71.6)	25 (28.4)	
College or more	216 (84.4)	40 (15.6)	
Employed at entrance into study, *n* (%)			0.04
Yes	193 (81.1)	45 (18.9)	
No	157 (44.9)	48 (51.6)	
Insurance, *n* (%)			0.4
Medicaid/none	216 (75.3)	71 (24.7)	
Commercial	134 (78.4)	37 (34.3)	
Subjective health rating scale,[d] median (range)	9.0 (1–10)	9.0 (5–10)	0.6
Planned pregnancy, *n* (%)			0.01
Yes	179 (51.7)	34 (37.4)	
No	167 (74.6)	57 (25.5)	
BMI,[e] *n* (%)			0.02
≤30	254 (73.8)	90 (26.2)	
>30	96 (84.2)	18 (15.8)	
Season at study entry, *n* (%)			0.6
April–September	170 (75.9)	54 (24.1)	
October–March	180 (77.9)	51 (22.1)	
Baseline 25(OH)D, nmol/L, mean ± SD (range)			
Total	59.5 ± 23.8 (6.0–172.5)	50.5 ± 25.1 (6.5–120.5)	0.001
Black	39.4 ± 18.6 (6.0–108.8)	37.4 ± 17.6 (6.5–87.8)	0.6
Hispanic	59.3 ± 20.0 (17.3–103.8)	54.7 ± 20.6 (23.0–95.3)	0.3
White	74.6 ± 20.2 (29.5–172.5)	68.8 ± 28.8 (23.3–120.5)	0.2

[a]Exited included patients who chose not to continue. It does not include those with pregnancy losses, those who became medically ineligible, or those who moved from the geographic area.
[b]Race/ethnicity as defined by the mother.
[c]Gestational age at enrollment based on last menstrual period; change in gestational age occurred in 11 cases at the time of the 20-week fetal ultrasound.
[d]Self-reported maternal health status rating from 1 (poor) to 10 (excellent).
[e]BMI = prepregnancy body mass index.

Compared with women who continued in the study until delivery, women who exited the study were more likely to be younger ($p = 0.01$), black ($p = 0.003$), of higher gravidity ($p < 0.0001$), less educated ($p = 0.01$), employed at entrance into the study ($p = 0.04$), with an unplanned pregnancy ($p = 0.01$), and with a BMI of less than 30 ($p = 0.02$). Baseline vitamin D status by ethnicity of those who completed versus those who exited also did not differ (see Table 2).

With regard to pregnancy losses, there were 8 women in the 400-IU group (baseline mean ± SD 16.5 ± 7.6 weeks, median 15.5 weeks, range 10.0 to 34.0 weeks), 5 in the 2000-IU group (baseline mean ± SD 17.2 ± 4.6 weeks, median 15.0 weeks, range 12.0 to 23.0 weeks), and 10 in the 4000-IU group (baseline mean ± SD 16.4 ± 6.3 weeks, median 16.0 weeks, range 9 to 32 weeks) who experienced a loss after enrollment into the study. The 25(OH)D level around or at the time of the loss did not differ by treatment group ($p = 0.8$) There were no statistically significant differences in mean gestational age at loss among the treatment groups ($p = 0.9$) or in the percent losses per treatment group ($p = 0.4$). When looking at baseline 25(OH)D

levels of women who delivered a live-born infant versus those who experienced a pregnancy loss, the mean levels were 57.8 ± 24.4 nmol/L versus 50.5 ± 23.3 nmol/L, but this did not reach statistical significance.

Among the 350 women who continued in the study until delivery, the median ratio of the number of study capsules taken to the number that should have been taken between the time of randomization and delivery was similar between the groups. Adherence to protocol was not statistically different between treatment groups: 69% (400-IU group), 68% (2000-IU group), and 69% (4000-IU group, $p = 0.9$).

Study outcomes

As shown in Table 3, the primary outcome—mean circulating 25(OH)D level one month prior to delivery and at delivery—was statistically different between treatment groups, with the highest mean level achieved in the 4000-IU group. Overall, the mean 25(OH)D level by dose group one month before delivery and at delivery and as chronic levels measured as the average from 20 to 36 weeks of gestation were significantly different between control and 2000 IU, control and 4000 IU, and 2000 and 4000 IU ($p < 0.0001$).

The secondary outcome measure of attaining a total circulating 25(OH)D level of at least 80 nmol/L at the time of delivery was met by 43 of 86 (50%) women in the 400-IU group, 63 of 80 (70.8%) in the 2000-IU group, and 68 of 83 (82%) in the 4000-IU group (Table 3), but there were 92 women with missing levels at delivery ($p < 0.0001$). Because of the high correlation ($r = 0.72$, $p < 0.0001$) between 1 month prior to delivery and delivery 25(OH)D values, 1 month prior to delivery values were used as a surrogate for delivery values for the women with missing delivery room values. When combined, 57 of 109 (52.3%) women in the 400-IU group, 93 of 117 (79.5%) in the 2000-IU group, and 94 of 112 (83.9%) in the 4000-IU group achieved a minimal circulating 25(OH)D level of at least 80 nmol/L around the time of delivery ($p < 0.0001$). Expressed as relative risk ratios, as shown in Table 4, there were significant differences between the 2000- versus 400-IU groups (relative risk [RR] = 1.52, 95% CI 1.24–1.86) and between the 4000- versus 400-IU groups (RR = 1.60, 95% CI 1.32–1.95), but there was not a significant difference between the 2000- and 4000-IU groups in this regard.

Vitamin D supplementation at various treatment doses given to our pregnant population had a variable effect on circulating levels of vitamin D_3 and its metabolites (Fig. 2*A*). Supplementation of vitamin D_3 at double the prior 1997 institute of Medicine (IOM) recommendation of 200 IU/d[40] and the current IOM estimated average requirement (EAR)[62] provided essentially no increase in circulating vitamin D_3 levels and only a minimal 5 ng/mL rise in circulating 25(OH)D levels over the duration of the study (Fig. 2*A*, *B*). Conversely, supplementing 2000 or 4000 IU/d vitamin D_3 had a profound effect on increasing both circulating levels of vitamin D_3 and 25(OH)D levels (Fig 2*A*, *B* and Table 5*A*, *B*). Figure 3*A* describes the substrate-product relationship in all patients between vitamin D_3 and 25(OH)D. The relationship is biphasic with respect to 25(OH)D production, requiring at least 10 ng/mL of circulating vitamin D_3 to saturate the vitamin D-25-hydroxylase.

Table 5*A*, *B* provides additional information with respect to circulating 25(OH)D concentrations analyzed by race, vitamin D dose, and stage of gestation. Clearly, race and duration of supplementation have profound effects on the circulating level of 25(OH)D attained. Black women lag at every time point and dose in relation to circulating 25(OH)D level. This is especially noticeable in the 400-IU group. In contrast, a greater proportion of black women achieved a 25(OH)D level of 80 nmol/L or greater by the second trimester in the 4000-IU group when compared with both the 400- and 2000-IU groups (Table 5*B*).

One of the most interesting biochemical findings in our study was the association between circulating $1,25(OH)_2D_3$ levels and of circulating 25(OH)D levels (Figs. 2*C* and 3*B*). In exploring the association between 25(OH)D and $1,25(OH)_2D_3$ levels, 25(OH)D level was found to have a direct influence on $1,25(OH)_2D$ levels throughout pregnancy ($p < 0.0001$). While the baseline $1,25(OH)_2D_3$ level in all groups at 12 weeks' gestation were not significantly different (Fig. 2*C*), within a few weeks, however, the circulating $1,25(OH)_2D_3$ levels became significantly

Table 3. Total Circulating 25(OH)D Concentrations (nmol/L) During Pregnancy

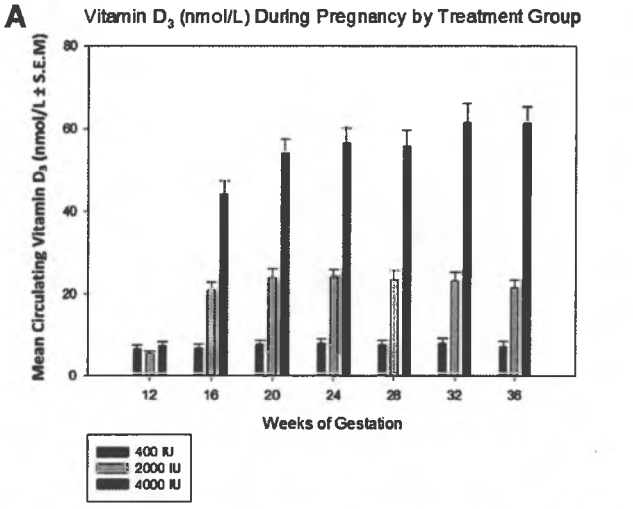
Measure	400-IU group	2000-IU group	4000-IU group	*p* Value
25(OH)D at baseline, mean ± SD	61.6 ± 27.1	58.3 ± 22.3	58.2 ± 21.8	0.5
Range	(6.0–172.5)	(14.0–115.3)	(11.8–109.3)	
25(OH)D 1 month before delivery, mean ± SD	79.4 ± 34.3	105.4 ± 35.7	118.5 ± 34.9	<0.0001
Range	(16.0–193.0)	(17.3–176)	(26.3–243.5)	
25(OH)D at delivery, mean ± SD	78.9 ± 36.5	98.3 ± 34.2	111.0 ± 40.4	<0.0001
Range	(12.5–159.5)	18.0–177.0	25.0–251.0	
25(OH)D, 20 to 36 weeks,[a] mean ± SD	79.1 ± 29.5	94.4 ± 26.1	110.8 ± 28.3	<0.0001
Range	(17.1–162.3)	(16.7–149.1)	(26.5–212.3)	
Achieved 25(OH)D level ≥ 80 nmoL at 1 month prior to delivery, *n* (%)	51 (50.0)	82 (73.9)	91 (82.0)	<0.0001
Achieved 25(OH)D level ≥ 80 nmoL at delivery, *n* (%)	43 (50.0)	63 (70.8)	68 (82.0)	<0.0001
Achieved 25(OH)D level ≥ 80 nmoL at 1 month prior to delivery or at delivery, *n* (%)	57 (52.3)	93 (79.5)	94 (83.9)	<0.0001
Infant birth 25(OH)D, mean ± SD	18.2 ± 10.1	22.8 ± 9.8	26.5 ± 10.3	<0.0001
Range	(2.4–48.4)	(3.6–47.9)	(2.4–52.0)	

[a]Mean value was the average 25(OH)D steady-state value obtained at visits between 20 and 36 weeks of gestation.

Table 4. Secondary Outcome: Achieving Total Circulating 25(OH)D ≥ 80 nmol/L Around Time of Delivery by Treatment Group

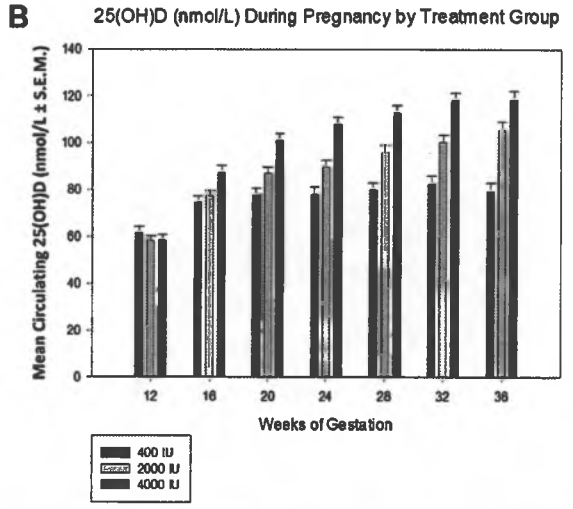
25(OH)D, nmol/L	2000 IU, *n* (%)	400 IU, *n* (%)	Risk ratio (95% CI)	Risk difference (95% CI)
≥80 nmol/L	93 (79.5)	57 (52.3)	1.5200 (1.2426–1.8594)	0.2719 (0.1530–0.3909)
	4000 IU, *n* (%)	400 IU, *n* (%)	Risk ratio (95% CI)	Risk difference (95% CI)
≥80 nmol/L	94 (83.9)	57 (52.3)	1.6049 (1.3183–1.9540)	0.3163 (0.2005–0.4322)
≥80 nmol/L	4000 IU, *n* (%)	2000 IU, *n* (%)	Risk ratio (95% CI)	Risk difference (95% CI)
	94 (83.9)	93 (79.5)	1.0559 (0.9340–1.1936)	0.0444 (–0.0555–0.1443)

Note: Vitamin D sufficiency was defined a priori as a total circulating 25(OH)D concentration of 80 nmoL (32 ng/mL) or greater. The following comparisons were made: 2000-IU group versus the 400-IU group, the 4000-IU group versus the 400-IU group, and lastly, the 4000-IU group versus the 2000-IU group. Risk ratios and risk differences were reported for each comparison with 95% CIs.



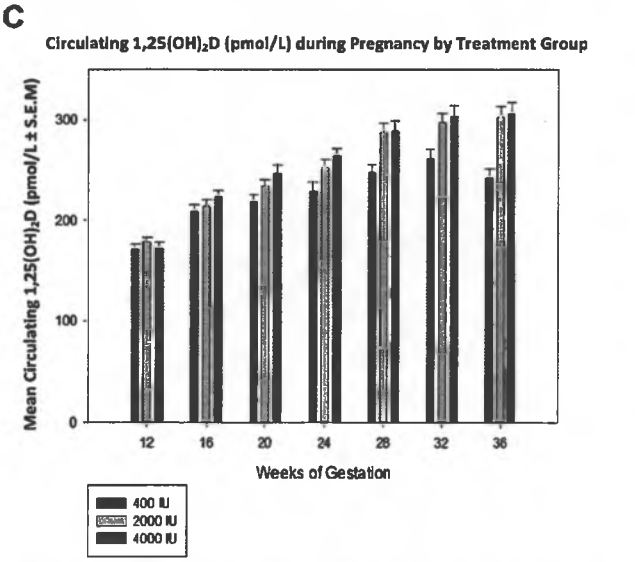
Statistical difference (p-value) in mean Vitamin D_3 between treatment groups at each week of gestation

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



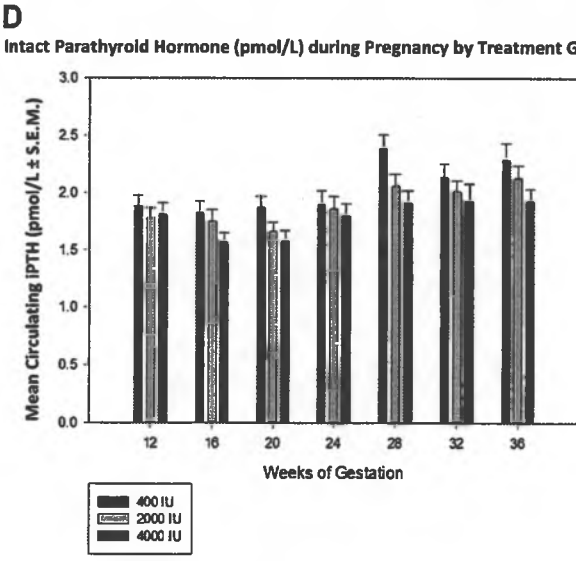
Statistical difference (p-value) in mean 25(OH)D between treatment groups at each week

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



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



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

Fig. 2. Circulating vitamin D, its metabolites, and intact PTH as a function of vitamin D_3 dose and time during pregnancy. (*A–D*) The mean (± SEM) circulating concentrations of vitamin D, 25(OH)D, $1,25(OH)_2D_3$, and intact PTH at defined time points during pregnancy.

Table 5. Circulating 25(OH)D and PTH Changes During Pregnancy by Treatment Group and Race/Ethnicity

A. Circulating 25(OH)D (nmol/L) by Trimester Stratified by Treatment Group

Treatment group	Baseline 25(OH)D, mean ± SD	Second trimester,[a] mean ± SD	One month prior delivery, mean ± SD
400 IU	61.2 ± 27.1	76.1 ± 27.5	81.2 ± 35.9
2000 IU	57.5 ± 22.4	84.2 ± 23.0	102.6 ± 36.4
4000 IU	59.8 ± 25.4	98.6 ± 27.3	114.2 ± 35.5
p Value	0.5	<0.0001	<0.0001

B. Circulating 25(OH)D (nmol/L) By Trimester Stratified by Treatment Group and Race/Ethnicity

	400 IU				2000 IU				4000 IU			
Characteristic	Baseline 25(OH)D, mean ± SD	Second trimester, mean ± SD	One month prior to delivery, mean ± SD	Δ Baseline to 1 month prior[b] (*p* value)	Baseline 25(OH)D, mean ± SD	Second trimester, mean ± SD	One month prior to delivery, mean ± SD	Δ Baseline to 1 month prior (*p* value)	Baseline 25(OH)D, mean ± SD	Second trimester, mean ± SD	One month prior to delivery, mean ± SD	Δ Baseline to 1 month prior (*p* value)
Black	37.3 ± 17.1	48.8 ± 21.1	49.4 ± 28.4	12.7(0.009)	41.0 ± 19.1	72.2± 28.4	91.2 ± 45.1	49.4 (<0.0001)	40.7 ± 20.1	81.0 ± 26.4	97.8 ± 42.4	57.4 (<0.0001)
Hispanic	59.1 ± 21.6	76.9 ± 21.7	79.5 ±30.3	20.3 (<0.0001)	59.2 ± 18.9	85.2 ± 16.8	102.1 ± 28.7	42.1 (<0.0001)	63.3 ±27.6	101.4 ± 28.2	121.1 ± 30.9	60.1 (<0.0001)
White	81.3 ± 23.8	95.2 ± 20.6	106.9 ± 26.4	25.0 (<0.0001)	71.9 ± 19.0	94.9 ± 18.3	115.7 ± 31.8	44.4 (<0.0001)	71.3 ± 17.3	109.8 ± 19.2	120.4 ± 29.7	50.4 (<0.0001)
p Value	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	0.02		<0.0001	<0.0001	0.008	

C. Intact PTH (pmol/L) by Trimester Stratified by Treatment Group

Treatment group	Baseline PTH, mean ± SD	Second trimester,[c] mean ± SD	One month prior to delivery, mean ± SD
Control	1.9 ± 1.0	1.9 ± 1.0	2.2 ± 1.3
2000 IU	1.8 ± 0.9	1.7 ± 0.9	2.1 ± 1.1
4000 IU	1.8 ± 1.1	1.6 ± 0.8	1.9 ± 1.1
p Value	0.5	0.1	0.1

D. PTH (pmol/L) by Trimester Stratified by Treatment Group and Race/Ethnicity

	400 IU			2000 IU			4000 IU		
Characteristic	Baseline PTH, mean ± SD	Second trimester, mean ± SD	One month prior, mean ± SD	Baseline PTH, mean ± SD	Second trimester, mean ± SD	One month prior, mean ± SD	Baseline PTH, mean ± SD	Second trimester, mean ± SD	One month prior, mean ± SD
Black	2.5 ± 1.2	2.6 ± 1.2	3.1 ± 1.8	2.1 ± 1.1	2.0 ± 0.9	2.3 ± 1.3	2.0 ± 0.9	1.9 ± 0.9	2.3 ± 1.1
Hispanic	1.8 ± 0.9	1.8 ± 0.7	2.0 ± 1.0	1.7 ± 0.9	1.8 ± 1.0	2.2 ± 1.1	1.8 ± 1.1	1.5 ± 0.7	1.7 ± 0.9
White	1.6 ± 0.9	1.4 ± 0.8	1.7 ± 1.0	1.6 ± 0.7	1.5 ± 0.7	1.9 ± 1.0	1.7 ± 1.2	1.6 ± 0.8	1.7 ± 1.3
p Value	0.001	<0.0001	0.0002	0.01	0.055	0.3	0.6	0.06	0.06

[a]Second-trimester mean value was the average 25(OH)D value obtained at visits between 16 and 24 weeks of gestation.
[b]Δ baseline to 1 month prior connotes the change from the baseline 25(OH)D level to the level achieved at 1 month prior to delivery.
[c]Second-trimester mean value was the average PTH value obtained at visits between 16 and 24 weeks of gestation.

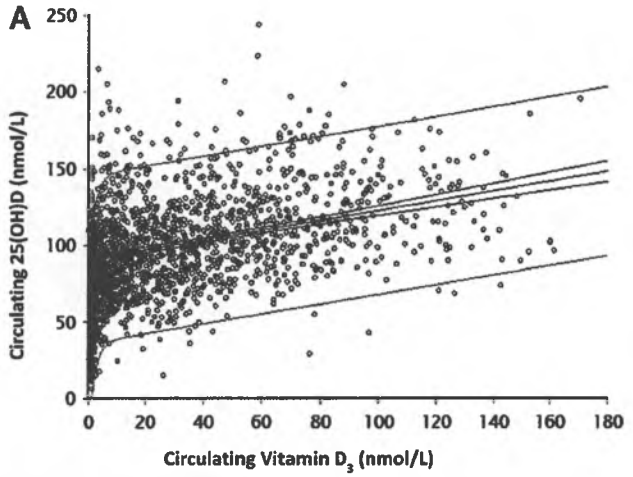


Fig. 3. Substrate-product relationships of vitamin D metabolites during pregnancy. (*A*) The relationship between circulating vitamin D to control the production of 25(OH)D during pregnancy. (*B*) The relationship of circulating 25(OH)D to control the production of $1,25(OH)_2D_3$ during pregnancy. All data points for all subjects in all groups were included in this analysis.

elevated in the 2000- and 4000-IU groups as opposed to the 400-IU group.

The relationship between these vitamin D metabolites is examined more closely in Fig. 3*B*. This figure clearly demonstrates a biphasic relationship between circulating 25(OH)D and $1,25(OH)_2D_3$, with circulating levels of 25(OH)D of at least 100 nmol/L (40 ng/mL) required to support maximum $1,25(OH)_2D_3$ output in the pregnant women. It is also worthy to note that circulating $1,25(OH)_2D_3$ levels at 12 weeks' gestation are approximately triple that of normal, nonpregnant female and normal male subjects, as reported previously[56] (Fig. 2*C*).

Figure 2*D* and Table 5*C*, *D* also display circulating intact PTH levels. The trend of PTH in all subjects was higher as the subjects progressed through pregnancy but was not significantly different by treatment group. Decreases in circulating PTH were observed if the levels attained were analyzed by race. The black group clearly had decreasing circulating PTH as circulating 25(OH)D levels increased (Table 5*C*, *D*).

Circulating levels of VDBP were measured in 80 selected subjects based on their circulating $1,25(OH)_2D_3$ levels, which ranged from 224.9 to 768.0 pmol/L at various stages of gestation. The average level of VDBP detected in these subjects was 5.45 ± 1.26 μmol/L, which represented a 39% increase over normal subjects. Further, using linear regression, no relationship was observed between circulating VDBP and $1,25(OH)_2D_3$ levels.

With respect to the effect of circulating 25(OH)D on either blood calcium or urinary calcium level, no significant effects were observed with one exception—that being the relationship between low circulating 25(OH)D and urinary calcium levels (Figs. 4*A*, *B* and 5). From Fig. 5, it would appear that approximately 75 nmol/L (30 ng/mL) of circulating 25(OH)D was required in the pregnant women to normalize urinary

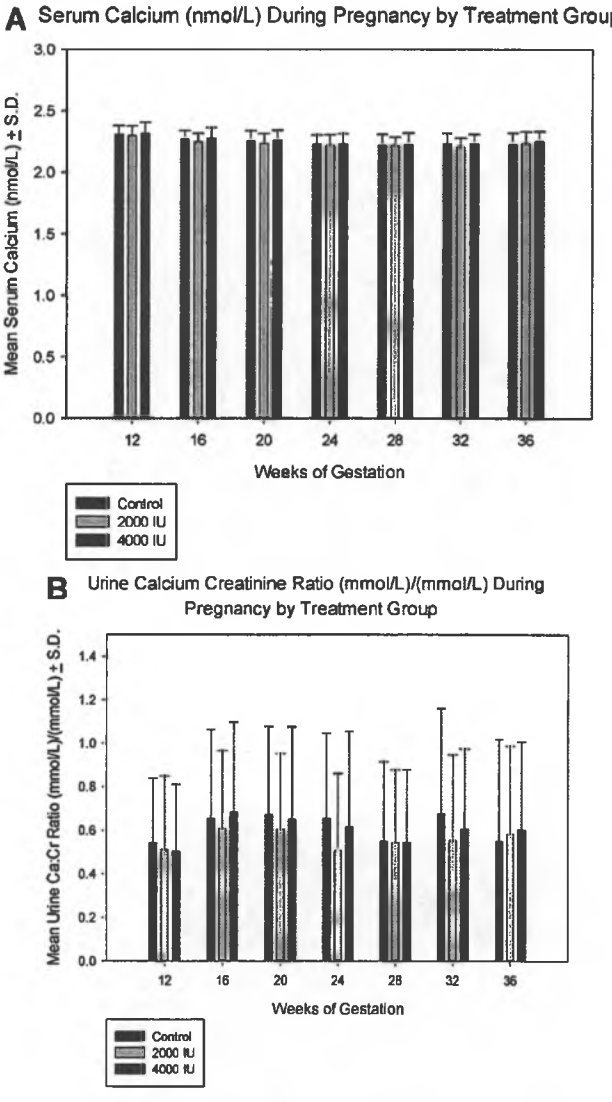


Fig. 4. Serum calcium and urinary calcium/creatinine ratio as a function of vitamin D_3 dose and time during pregnancy. (*A*, *B*) the mean (± SD) serum calcium and urinary calcium/creatinine ratio at defined time points during pregnancy.

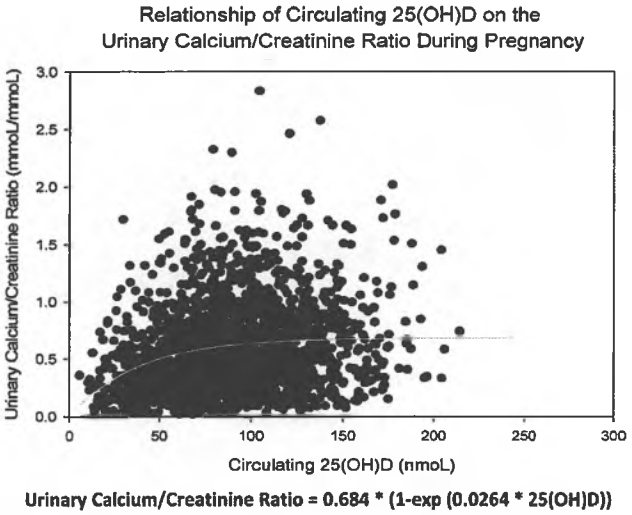


Fig. 5. Relationship of circulating 25(OH)D on the urinary calcium/creatinine ratio during pregnancy. All data points are included for all study patients. Urinary calcium and urinary creatinine were measured in mmol/L. Ratio was calculated from measurement of urinary calcium (mmol/L) divided by measurement of urinary creatinine (mmol/L).

calcium excretion. Above that threshold, 25(OH)D appeared not to influence urinary calcium and subsequent excretion.

Throughout the study, there were no statistically significant differences between groups on any safety measure: serum calcium, creatinine, and phosphorus and urinary calcium/creatinine ratios (p value not significant [pNS] between groups). Review of adverse events by the DSMC showed that not a single adverse event in this trial was attributed to vitamin D supplementation or circulating 25(OH)D levels. There was one safety measure stop implementation: In the 4000-IU group, one woman with a baseline circulating 25(OH)D level of 29.3 nmol/L (13.3 ng/mL) increased to 233.3 nmol/L (93.3 ng/mL) at visit 2. Her follow-up circulating 25(OH)D level at the return visit prior to stopping supplementation had decreased to 66.6 ng/mL. Although her urinary calcium/creatinine ratio and all serum biochemical indices were within normal limits, the woman ceased supplementation per protocol. Two additional women met upper threshold criteria at the time of delivery; both had commenced sunbathing during the weeks prior to delivery; no toxicity by any parameter in either mother or baby was found.

Mode of delivery and neonatal characteristics by maternal treatment group are found in Table 6. There were no differences between the groups in terms of gestational age at delivery or birth weight. In addition, there were no significant differences in level of care (newborn nursery versus higher level of care, ie, level 2 or neonatal intensive care admission) or increased adverse outcomes of pregnancy related to maternal vitamin D intake. There were several differences, however, in terms of neonatal vitamin D status by treatment group. Neonatal 25(OH)D was significantly correlated with maternal 25(OH)D overall, 1 month prior, and at delivery ($r^2 = 0.6$, odds ratio [OR] = 0.50) and was significantly different by treatment group: 45.5 ± 25.3 nmol/L (18.2 ± 10.1 ng/mL, control), 57.0 ± 24.5 nmol/L (22.8 ± 9.8 ng/mL, 2000-IU group), and 66.3 ± 25.8 nmol/L (26.5 ± 10.3 ng/mL, 4000-IU group; $p < 0.0001$). By treatment group, using IOM guidelines for sufficiency [total circulating 25(OH)D $\geq$ 50 nmol/L or 20 ng/mL],[62] 31 of 78 (39.7%) neonates in the 400-IU group, 53 of 91 (58.2%) in the 2000-IU group, and 66 of 84 (78.6%) in the 4000-IU group had a cord blood/neonatal 25(OH)D level in the sufficient range ($p < 0.0001$).

Discussion

In this randomized, controlled trial of vitamin D supplementation during pregnancy involving a diverse group of women living at latitude 32°N, those women randomized to 4000 IU/day compared to those receiving 400- or 2000 IU/day experienced improved vitamin D status throughout pregnancy, 1 month prior to delivery, and improved vitamin D status in their offspring at birth. Irrespective of race and ethnicity, this improvement in vitamin D status was achieved without any evidence of

Table 6. Characteristics at Delivery by Vitamin D Supplementation Group

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
Mode of delivery[a]: 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, any type	71 (74.7%)	85 (79.4%)	90 (85.7%)	0.15
Primary C/S	24 (25.3%)	22 (20.6%)	15 (14.3%)	
Gestational age (weeks) at delivery, mean ± SD	38.6 ± 2.2	38.8 ± 1.8	39.1 ± 1.8	0.17
Birth weight (g) at delivery, mean ± SD	3221.8 ± 674.9	3360.1 ± 585.0	3284.6 ± 597.6	0.23
Admission to level II or III, n (%)	12 (10.8%)	14 (11.5%)	11 (9.4%)	0.9

Delivery Characteristics by Vitamin D Supplementation Group:

[a]Mode of delivery was categorized a priori as either a vaginal delivery (defined as spontaneous vaginal delivery or assisted vaginal delivery [which included use of forceps or vacuum extraction]) or cesarean section (C/S; further subdivided as cesarean following labor, cesarean without labor, and repeat elective cesarean). Primary cesarean section included women who had undergone a cesarean section with or without labor for either a maternal or fetal indication and did not include women who underwent a repeat, elective cesarean section.

hypervitaminosis D or an increase in adverse events during pregnancy and with optimization of 25(OH)D and $1,25(OH)_2D_3$. From the standpoint of enzyme kinetics, this simply means that in the case of vitamin D being converted to 25(OH)D and subsequently to $1,25(OH)_2D_3$, enzyme saturation is occurring; that is, reaction rates are moving from first-order to zero-order enzyme kinetics. In simple terms, this means that an appropriate amount of substrate is being supplied to produce maximum product, that is, 25(OH)D and $1,25(OH)_2D_3$; as such, no substrate "starvation" is occurring.

At no point in human nutrition is it more critical to ensure adequate nutrient intake than during the state of pregnancy. Folate intake during pregnancy and its role in the development of neural tube defect serves as a stark example.[63,64] The limited clinical investigation into meaningful dietary vitamin D supplementation during pregnancy can be traced back to post–World War II Britain. Because of the British experience with idiopathic infantile hypercalcemia attributed to hypervitaminosis D, an inaccurate association occurred that had a profound effect on the potential of vitamin D supplementation not only during infancy but also during pregnancy. In 1963, Black and Bonham-Carter[65] recognized that the elfin facies observed in patients with severe idiopathic infantile hypercalcemia resembled the peculiar facies observed in patients with supravalvular aortic stenosis (SAS) syndrome. By 1966, vitamin D was viewed by the medical community as the cause of SAS syndrome.[66,67] With the advent of molecular genetics, the children with SAS syndrome were discovered to have Williams syndrome, an example of unipaternal disomy, with abnormal vitamin D metabolism.[68–75]

The perception that vitamin D can inflict harm during pregnancy still lives on today because many obstetrical specialists are afraid to undertake vitamin D repletion during this period. Research efforts in this area were further hampered when in 1997 the Institute of Medicine (IOM) issued guidelines that defined the adequate intake (AI) for vitamin D during pregnancy to be 200 IU/d, with intakes greater than 2000 IU/d causing potential harm.[40] Recently, the IOM issued new guidelines with respect to pregnant women that define the estimated average requirement (EAR) and recommended dietary allowance (RDA) to be 400 and 600 IU/d, respectively. The IOM also increased the tolerable upper intake limit (UL) to 4000 IU/d.[62] These new guidelines, with the exception of the UL, are based on old data because limited new data exist. The result of prior and current guidelines is that most prenatal vitamins contain only 400 IU of vitamin D. In our experience, many of today's practicing obstetricians are unaware of the vitamin D content in prenatal vitamins or have a fear of administering additional vitamin D supplements to pregnant women.

Our study was based on two previous vitamin D supplementation studies in nonpregnant adults that appeared to be safe.[48,58] Prior to undertaking the NIH-funded study described here, however, we had to obtain an Investigational Drug Number from the FDA, which entailed writing a complete investigational drug application. This was required by the FDA because we proposed using a vitamin D_3 dose of 4000 IU/d, 20 times the AI and twice the safe limit put forth by the IOM in 1997[40] but currently put forth as the UL.[62] Thus, our study is the first one to test this current UL in pregnant women.

The only known avenue of vitamin D toxicity is manifested through hypercalcemia and hypercalciuria,[76] neither of which was observed in our ramdomized, controlled trial (RCT). In fact, our Data and Safety Monitoring Committee concluded that not a single adverse event in this RCT could be attributed to vitamin D intake. Hypervitaminosis D is largely arbitrarily defined as circulating levels of 25(OH)D that exceed 375 nmol/L (150 ng/mL), a level we never attained with our dosing regimen. As has been observed in other human supplementation studies, the conversion of vitamin D to 25(OH)D appears to be controlled.[77] Further, it has been known for decades that during pregnancy $1,25(OH)_2D_3$ levels become extremely elevated.[78,79] This increase in circulating $1,25(OH)_2D_3$ levels has in particular been attributed to an increase in the serum vitamin D–binding protein (VDBP) that would regulate the amount of "free" $1,25(OH)_2D_3$ available in the circulation.[79] While this rise in VDBP during pregnancy has been shown to be 46% to 103%, depending on the assay employed,[80] it cannot account, however, for the nearly three- to fourfold increase in circulating $1,25(OH)_2D_3$ observed in our study. Bikle and colleagues[81] clearly demonstrated that free $1,25(OH)_2D_3$ levels are increased during pregnancy despite the significant increase in VDBP levels. We were unable to measure "free" circulating levels of $1,25(OH)_2D_3$ in our subjects, but our data agree with those of Bikle and colleagues in that no relationship was observed during pregnancy between circulating VDBP and "total" circulating $1,25(OH)_2D_3$.[81] New data from our study suggest that a circulating 25(OH)D level of approximately 100 nmol/L (40 ng/mL) is required to optimize production of $1,25(OH)_2D_3$ during human pregnancy through renal and/or placental production of the hormone (Figs. 2*C* and 3*B*). It is also of great interest that production of circulating $1,25(OH)_2D_3$ in the fetus is linked directly to circulating 25(OH)D.[10]

Clearly, vitamin D metabolism is greatly altered during pregnancy, and pregnancy itself is the primary driver for these extraordinary circulating $1,25(OH)_2D_3$ levels. From our data, it is evident that production of $1,25(OH)_2D_3$ is really not under the control of the classic regulators of calcium, phosphorus, and PTH. The dramatic rise in maternal circulating $1,25(OH)_2D_3$ following conception is remarkable for many reasons: By 12 weeks of gestation, maternal circulating $1,25(OH)_2D_3$ levels are already triple those of a nonpregnant female (Fig. 2*C*). From that point in gestation, the $1,25(OH)_2D_3$ levels rise much higher and are driven by substrate—25(OH)D—availability (Fig. 3*B*). This substrate dependence of $1,25(OH)_2D_3$ production is never observed in normal human physiology driven by classic calcium homeostasis.[10,82,83]

Another remarkable factor in pregnant women is how they can attain supraphysiologic levels of $1,25(OH)_2D_3$, sometimes exceeding 700 pmol/L in our study, and never exhibit hypercalciuria or hypercalcemia. These tremendous circulating levels of $1,25(OH)_2D_3$ during pregnancy are possibly of placental origin or from the renal 1-α-hydroxylase that would have to be uncoupled from feedback control and for reasons other than maintaining calcium homeostasis. The second scenario is most likely because women with nonfunctional renal 1-α-hydroxylase and normal placental function fail to increase circulating $1,25(OH)_2D_3$ during pregnancy.[84] The increased levels of $1,25(OH)_2D_3$ may be due to

the methylation of the catabolic *CYP24A1* placental gene.[85] It is possible that calcitonin may be a contributor to this process in that calcitonin rises during pregnancy,[86] is known to stimulate the renal *1-α-hydroxylase* gene independent of calcium levels,[87,88] and also protects by opposing hypercalcemia.[89] Another possible stimulator of 1-α-hydroxylase during pregnancy is prolactin.[90] If prolactin were a major contributor, however, the effect should continue into lactation, which we do not see, and would be accompanied by elevated circulating $1,25(OH)_2D_3$ levels, which also are not seen.[91] Further, the physiologic function of this altered vitamin D metabolism may be related to increased reliance on innate immune function during pregnancy, as well as decreased adaptive immune responses,[7,8,10,92] protecting the newborn from respiratory infection and subsequent wheezing[93,94] and possibly epigenetic alterations in invariant natural killer (NK) T cells, which can lead to increased autoimmune disease prevalence.[95,96] As supported by this and prior studies, it is important to remember that for cord blood to attain a 25(OH)D level of 50 nmol/L, the maternal 25(OH)D level would need to be at least 80 nmol/L.[97]

Our data also suggest that a circulating level of approximately 75 nmol/L (30 ng/mL) of 25(OH)D is required to normalize calcium excretion into the urine. Interestingly, this value is virtually identical to the value obtained by Heaney and colleagues with respect to the equilibration of intestinal calcium absorption.[98] This increased level of circulating 25(OH)D in the pregnant woman also appears to reduce circulating PTH, especially in black subjects. It is also important to compare our study results with respect to two recent reports dealing with vitamin D supplementation during pregnancy.[62,99] The IOM report recommends a vitamin D intake of 400 to 600 IU/d and states that this level can be obtained solely from the diet. Further, this intake level would be sufficient to meet their circulating 25(OH)D target of 20 ng/mL (50 nmol/L).[62] Even using this conservative 25(OH)D level, the IOM recommendation would have left more than 50% of our total cohort and more than 80% of black women in the cohort deficient at study entry. The Endocrine Society's recommendation of a daily vitamin D intake of 1500 to 2000 IU and target 25(OH)D level of greater than 30 ng/mL (75 nmol/L)[99] is more sound advice yet is still conservative compared with our study results. It must be pointed out that the purpose of the IOM report was to guide food manufacturers and fortifiers and is not intended to guide clinical practice.[62] On the other hand, clinical practice guidance is precisely the purpose of the Endocrine Society's recommendations.[99]

This study has certain limitations. This study was conducted at a southern latitude, and therefore, the vitamin D requirements of women living at more northern latitudes could be greater. While women with preexisting hypertension and diabetes were excluded from the study, these women may be at greater risk of vitamin D deficiency and therefore may receive the greatest benefit from vitamin D supplementation of 4000 IU/d. Because of safety concerns, women were not allowed to remain in the study if their total circulating 25(OH)D level rose above 225 nmol/L. There were three women who attained this threshold, none of whom had any associated hypercalciuria or hypercalcemia. Lastly, owing to safety concerns that surrounded the use of 4000 IU of vitamin D supplementation during pregnancy, the study was designed to begin supplementation starting at the twelfth week of gestation, beyond the period of early organogenesis. Hence we cannot ensure the safety before the twelfth week of gestation. With regard to vitamin D intake during pregnancy, it is interesting that our study largely confirms the observations of Obermer in England more than 60 years ago.[100] Obermer's suggestions largely were ignored because of greatly flawed associations between vitamin D and SAS syndrome.[65,66,101] The data in our paper put us back on the path suggested by Obermer with respect to vitamin D intake during pregnancy. Additional studies will be necessary to ascertain safety of 4000 IU/d of vitamin D supplementation before the twelfth week of gestation.

Conclusions

In summary, starting at 12 to 16 weeks of gestation, vitamin D supplementation with 4000 IU/d was most effective in achieving vitamin D sufficiency throughout pregnancy, 1 month prior to delivery, and at delivery in a diverse group of women and their neonates without increased risk of toxicity. These findings suggest that the current vitamin D EAR and RDA for pregnant women issued in 2010 by the IOM[62] should be raised to 4000 IU of vitamin D per day so that all women, regardless of race, can attain optimal nutritional and hormonal vitamin D status throughout pregnancy.

Disclosures

BWH serves as a consultant for Diasorin, Inc. (Stillwater, MN, USA). All the other authors state that they have no conflicts of interests.

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authors contributed to revising the manuscript content. Approving final version of manuscript: All the authors approved the final version of the manuscript. TCH and ME take responsibility for the integrity of the data analysis.

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PROTECT OUR CHILDREN NOW!

Moving Proven Research Into Practice—NOW!

A Public Health Action Project

Improve Prenatal and Newborn Health Outcomes

Education - Measurement - Tracking - Public Health Action

Project Manual

Revised: September 23, 2013

PROTECT OUR CHILDREN NOW!

This manual is prepared for the Protect Our Children *NOW*! Project.

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PROTECT OUR CHILDREN NOW!
A Vitamin D-action Project of GrassrootsHealth
A Public Health Promotion Organization

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PROTECT OUR CHILDREN NOW!

Table of Contents

1.0 Project description

1.1 Purpose of Protect Our Children *NOW!* (POC)

Protect Our Children *NOW!* is a public health project whose purpose is to provide education and vitamin D testing to pregnant mothers (and subsequently, their infants) to help individuals manage their pregnancy health outcomes with regard to vitamin D status. The project will acquire health information with respect to the outcomes of the pregnancies of the individuals enrolled, i.e., such variables as preterm births, Caesarean sections, preeclampsia, low weight for gestational age, and similar outcomes, and analyze the outcomes in relation to achieved 25(OH)D status. The project is a public health intervention based on the Hollis, Wagner randomized trial (see reference) which showed that a serum level of at least 40 ng/ml was a preventive factor that predisposed the mother and fetus to decreased prenatal and postnatal diseases. This report (Am J Obstet Gynecol. 2012 Nov 3), showed a 50% reduction in preterm births with the mother's vitamin D level at approximately 40 ng/ml, among other substantial health benefits. Results will be taken to public health officials and translated to a larger population to both substantiate the results as well as to help all pregnant women have safer pregnancies, translating research into practice.

1.2 Overview of the GrassrootsHealth D*action Project

Protect Our Children *NOW!* (POC) is a project of the GrassrootsHealth D*action effort. GrassrootsHealth (GRH), as a non-profit public health organization, is committed to improving vitamin D status worldwide.

D*action is an international public health project started by GRH to help solve the vitamin D deficiency epidemic. It currently involves over 10,000 individuals world-wide. Its principal method consists of two efforts: 1) providing educational material concerning the benefits of adequate vitamin D status, and 2) providing information about the participants' own vitamin D status, thereby allowing participants to adjust their vitamin D intake to achieve desired levels. The database which GRH has created contains anonymized health status information provided by the enrollees. This database is available to competent scientists for the testing of various hypotheses.

As a community-based subset of this larger D*action project, POC is focused on pregnant women and their infants. The intervention, as with the larger project, consists of the provision of educational materials and information about the participants' own vitamin D status, together with gathering anonymized health status information from the participants.

2.0 Overview of the Protect Our Children *NOW!* Project Design

2.1 Participants

The project will involve 500 women per sponsored community who are enrolled between the 12^{th} and 16^{th} week of pregnancy.

2.2 Procedure

Enrollment consists of the completion of a health questionnaire (including the consent to participate and to use the de-identified data for research purposes) and a serum 25(OH)D blood spot test kit. Follow-up questionnaires and serum 25(OH)D blood spot test kits will then be provided to be completed each 10 weeks of pregnancy (for a total of 3 tests) and once for the newborn at birth. The infant test is expected to be obtained from the cord blood at birth. Health outcomes will be tracked and measured throughout pregnancy and in the newborns. Educational tools and material will be provided to all participants on vitamin D and health.

2.3 Communications

Reminders (both phone and email) to complete correlating online health questionnaires will be provided in conjunction with each test. An on-site Program Facilitator will be available to participants in person should additional assistance be needed.

2.4 Outcomes Measured

Hard pregnancy outcomes such as pre-term birth, low birth weight, C-section, preeclampsia, infection and other pregnancy complications will be acquired. There is an extensive online questionnaire for data gathering that will be used with the pregnant women. It includes all the conditions that the Hollis, Wagner study used including many comorbidities of pregnancy as well as other health outcomes. We will be tracking intake of vitamin D and 25(OH)D serum levels and looking for any association between achieved 25(OH)D level and pregnancy outcomes. Additionally, outcomes in project participants will be contrasted with historical data from the same community.

A summary of what will be measured is shown in the table in Section 6.0 (*What we will be Tracking*).

2.5 Inclusion Criteria

This project will enroll:

1. Any woman who is 12-16 weeks pregnant is able to enroll in the study for vitamin D testing and education. Their newborns will also be included.
2. Offspring of the enrolled women.
3. An option of adding women to the project at a preconception stage is available.

2.6 Enrollment

Individuals will be recruited through referrals made by service providers and clinics involved in the POC program in the sponsored community.

Participants will be enrolled after they logon to the website, verify that they qualify and give consent, create their enrollment and enter data on a health questionnaire. They will then receive a 25(OH) D test, generally in the mail or at their service provider's location.

Informed consent is obtained through an IRB (Institutional Review Board) approved process utilizing online forms at enrollment, and a consent form for the newborn participants' birth location medical records, to be completed at birth.

2.7 Dismissal Process

Individuals who have enrolled in the POC program may be dismissed from further participation in the program under the following circumstances:

1. If the first prenatal 25 (OH)D blood spot test and/or health questionnaire are not completed and received by 20 weeks gestation.
2. If the second prenatal 25(OH)D blood spot test and/or the second health questionnaire are not completed and received by 30 weeks gestation.
3. If the third prenatal 25(OH)D blood spot test and/or the third health questionnaire are not completed and received by four weeks postpartum.
4. If the newborn 25(OH)D blood spot test and/or the newborn health questionnaire are not completed and received by two weeks of age.
5. If pregnancy is terminated, or if the participant suffers from miscarriage, stillbirth, or other loss of pregnancy.
6. If calcium levels too high? If other signs? Conditions? By order of the doctor?? (need feedback from Wagner/Heaney)

In the case of any of the above, the participant will be notified of their dismissal from the program, will be deactivated from the system, and will not receive additional 25(OH)D blood spot tests or communications regarding the program.

2.8 Projected Intervention Dates

This is a population study (a concurrent cohort study, not a clinical trial) which is aimed at providing continuous education about vitamin D and serum 25(OH) D testing to individuals so that they can decide for themselves what actions to take. The intervention is an intensive education program stressing the importance of vitamin D during pregnancy, along with other proven health factors such as proper exercise, diet and supplementation, as well as feedback in the form of actual vitamin D status (from the blood spot analysis).

Testing of serum 25(OH) D concentrations will be done at 12-14 weeks, 22-26 weeks and 32-36 weeks of the pregnant mother as well as of the infant at delivery. Both the pregnant women and their healthcare practitioners will be provided educational materials, videos and tracking tools, and will have access to a support service person via phone.

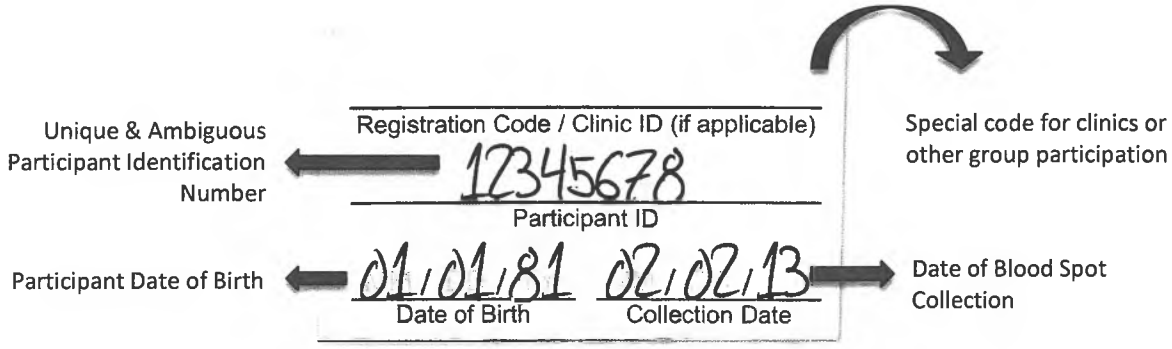
2.9 Duration of the Project – Phase 1

The approximate time from start to finish is about 24 months. The pregnant women are expected to all be enrolled within the first 10-12 months of the project and the last 9+ months will be data analysis, creation of a publication and submission to a journal. There will be the option for the women to continue their participation as well as having the child participate.

2.10 Sample/Data Identification & Handling

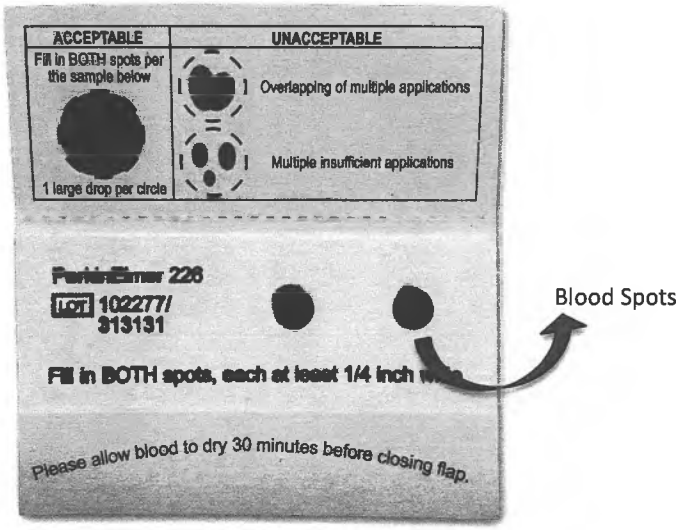
All test samples are provided from the participants with their unique participant ID on the blood spot sample card. The participant ID is assigned by the system at initial sign-up. This coding system allows for anonymous processing at the contracted laboratory.

Back of Blood Spot Specimen Card



The participants must provide blood spots that are at least ¼ inch or 6 mm in size each, and which penetrate the blotter paper, in order to be adequately processed and analyzed.

Interior of Blood Spot Specimen Card



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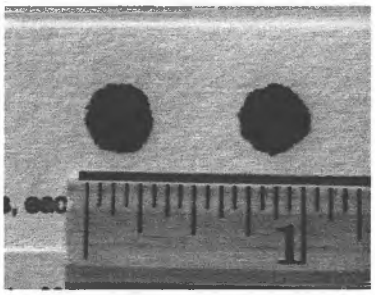
Blood Spots must be at least ¼ inch or 6mm Wide



Completed serum 25(OH) D blood spot test cards are sent directly from the participant to GrassrootsHealth and then processed at a contracted laboratory that adheres to the testing criteria established by the NIST (National Institute of Standards Technology).

The data from questionnaires are recorded each time a blood spot test is completed via the online entry system, MyOWNHealth (see Section 5.1), which could be at a clinic or used by a participant at home. The specifics of where the data are gathered are to be worked out with different community areas. Online and smart-device tools will also be available to individual participants and clinics to assist in and promote accurate tracking.

Other items clinics and/or participants will have the option to track include nutrient intake, exercise, lifestyle habits and other regular daily activities.

3.0 Project Kit Contents

The contents of each serum 25(OH)D blood spot test kit will provide all the necessary materials needed to complete the test safely at home. The vitamin D test requires that the participant use a self-loaded lancet to prick a fingertip to get blood to drop on a card. Enclosed in the kit are an alcohol swab, gauze, band aid, the bloodspot card, a return envelope and instructions.

The initial 25(OH) D blood spot test kit will be sent upon completion of registration, the online questionnaire and the consent forms. Follow-up test kits will be sent automatically with prompts for the participant to complete the corresponding online health questionnaire.

Serum 25(OH) D Test Kit Contents:

- 1 Instruction sheet
- 1 FAQ
- 1 POC tri-fold card
- 1 business card
- 1 addressed return envelope
- 1 blood spot card
- 1 gauze pad
- 1 alcohol prep pad
- 1 Band-Aid
- 2 lancets

Each test kit is associated with an online health questionnaire, to be completed within the same time frame. The Test Kit contents for the pregnant participants are the same as those for the newborns. The pregnancy health questionnaires and newborn heath questionnaire, to be completed online at the time of testing, are provided as a separate reference document.

4.0 Summary of Enrollment & Participation

4.1 Summary - Project Components

Online Registration
Consent to Participate

For the participant to complete
Online Health Questionnaire via MyOWNHealth
Medical Records Release Form
Consent to Notify Health Care Provider of Results (optional)

Once the above is completed
Shipment of Serum 25(OH)D Test Kit to Participant
Full access to MyOWNHealth Educational Tool and other Support Materials (see Section 5.1)

*Responsibilities of the **project coordinators**:*

1) Ongoing prompts and communications regarding completion of questionnaires, tests, and consent items
2) Ensuring eligibility of participants
3) Shipment of test kits to participants
4) Verbal and online assistance to participants if needed
5) Assisting with additional educational opportunities and materials

4.2 Summary – Timeline & Steps

Participant Enrollment Initiation & Completion

12-16 weeks gestation
Registration
Consent
Online Pregnancy Health Questionnaire 1
Serum 25(OH) D Test 1

22-26 weeks gestation
Online Pregnancy Health Questionnaire 2
Serum 25(OH) D Test 2

32-36 weeks gestation (or at birth if birth occurs first)
Online Pregnancy Health Questionnaire 3
Serum 25(OH) D Test 3

At Birth
Hospital Records Release Form
Online Newborn Health Questionnaire
Newborn Serum 25(OH) D Test

Data analysis
Creation of publications and journal submissions
Public action

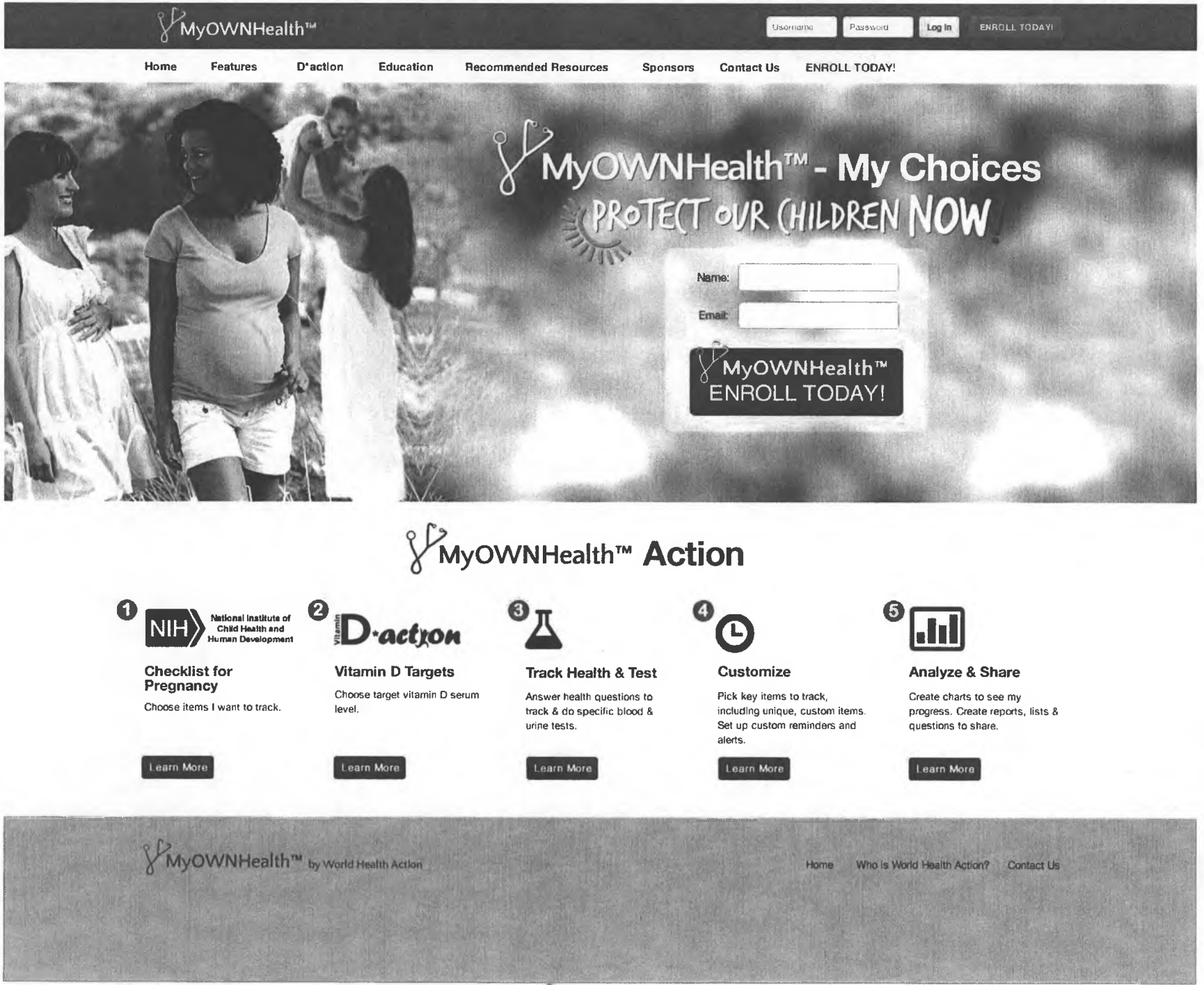
5.0 Participant Engagement

5.1 The MyOWNHealth™ Application

The MyOWNHealth™ Education System is a suite of applications for self-health management for individuals enabling personalized information, analysis and tracking of targeted health outcomes and changes by the end-user. Medical Practices can use the educational system modules for its clients to help the clients understand/learn about what health choices are recommended and to enable them to choose and track results, and learn how certain practices influence the outcomes. The end results are better informed and consequently healthier patients and, a more detailed compilation of health factors and outcomes.

This system is free to any participant of the Protect Our Children NOW project.

All questionnaire and health data is collected from the participants through the MOH application.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MyOWNHealth™ Action
1
NIH
National Institute of Child Health and Human Development
Checklist for Pregnancy
Choose items I want to track.
Learn More
2
Vitamin D-actyon
Vitamin D Targets
Choose target vitamin D serum level.
Learn More
3
Track Health & Test
Answer health questions to track & do specific blood & urine tests.
Learn More
4
Customize
Pick key items to track, including unique, custom items. Set up custom reminders and alerts.
Learn More
5
Analyze & Share
Create charts to see my progress. Create reports, lists & questions to share.
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MyOWNHealth Features

Track and Chart YOUR Health Stats

- Choose from the NICHD provided checklist of health factors to track
- Add additional custom health factors to track
- Generate and view charts of your health stats
- Set goals for any health factors you are tracking

A Personal Health System

- Set up custom reminders and alerts
- Add personalized notes when updating health stats
- Calendar for viewing & managing upcoming appointments and reminders
- Choose only items YOU want to track

Learn, Analyze & Share

- Compare multiple health factors with graphs to look for relationships
- Print customized reports with graphs of your health factors to provide to your physicians
- Browse our library of additional resources for information regarding health items you are currently tracking

6.0 Questionnaires

A copy of each online health questionnaire is provided as a separate reference document.

Instructions: how to correctly complete the forms

The health questionnaires and consent forms have been developed to be entered directly into a computer-based electronic database through the MyOWNHealth application system, with exception of the Medical Records Release Form.

Forms that will be completed directly onto the computer are listed below:
Online Participant Registration
Research Consent Form
Pregnancy Health Questionnaire 1, 2 and 3
Newborn Health Questionnaire

Forms that will be paper-based are listed below:
Medical Records Release Form

Information for the project coordinator:
Each questionnaire will have the name of the questionnaire, date of each entry, and the participant identification number.

Identification Number: The participant ID is assigned to each participant automatically by the electronic database at initial sign-up and enrollment. Each participant ID consists of a set of 8 numbers and is included on each specimen sent to the lab for processing.

7.0 What we will Track

	PREGNANCY	NEWBORN
Gender	X	X
Ethnicity	X	X
Weight	X	X
Height	X	X
BP	X	X
Pregnancy Status	X	X
Expected date of delivery	X	
Pregnancy History: # Pregnancies, # Live Offspring, complications, abortions, miscarriages	X	
Conditions of Pregnancy: Date of Dx, Hospitalization, Medication	X	
Gestational diabetes	X	
Pregnancy-induced hypertension	X	
Pre-eclampsia	X	
Eclampsia	X	
HELLP Syndrome	X	
Pre-term labor	X	
Infection: UTI, BV, GBS, Other	X	
Delivery Method	X	
Spontaneous vs/ Induced	X	
Induction Method	X	
Complications during Labor	X	
Failure to Progress	X	
Cephalopelvic Disproportion	X	
Fetal Decelerations / Fetal Distress	X	
Infection	X	
Placental abruption	X	
Other	X	

	PREGNANCY	NEWBORN
Gestation Time	X	X
Number of Offspring	X	
Health Status of Newborn at Birth		X
Living vs/ Stillborn		X
Complications		X
NICU Admission, Level, Reason		X
Preterm Birth		X
Respiratory Distress Syndrome		X
Newborn Septicemia		X
Transitory Tachypnea		X
Suspected Infection		X
Other		X
APGAR Score		X
Gestational Age: EDC, Exam		X
Breastfeeding Status		X
Formula Intake		X
Personal Health History	X	
Cancers: Breast, Colon, Prostate, Ovarian, Melanoma, Other	X	
Diabetes: Type 1, Type 2	X	
Multiple Sclerosis	X	
Hypertention	X	
Pneumonia	X	
Heart Attack	X	
Stroke	X	
Alzheimers	X	
Angina Pectoris	X	
Celiac Disease	X	
Chronic Fatigue	X	
Eczema or serious rash	X	
Preeclampsia	X	
Fibromyalgia	X	
Gluten Intolerance	X	
Kidney Failure	X	
Kidney Stones	X	

	PREGNANCY	NEWBORN
Lactose Intolerance	X	
Myasthenia Gravis	X	
Parkinsons	X	
Other	X	
STDs: Chlamydia, Gonorrhea, Herpes, HIV, Syphilis, Warts	X	
Drug Allergies	X	
Recent OTC Medication Use	X	
Surgical History	X	
Falls	X	
Broken Bones	X	
Colds	X	
Flu with symptoms	X	
fever	X	
muscle pains	X	
headaches	X	
weakness	X	
upper respiratory	X	
gastrointestinal	X	
Pain: location, rating, reason	X	
Milk Intake	X	
Vitamin D Intake: Amount, Brand, Type	X	X
Vitamin A Intake	X	
Calcium Intake	X	
Additional Supplement Use	X	
Sun Exposure: clothing, sunscreen, time of day	X	
Indoor Tanning	X	
Vacations: location, time of year	X	
Physical Activity: amount, indoor vs/ outdoor, mild vs/ moderate vs/ strenuous	X	
Smoking: current, history, second hand	X	
Alcohol Intake	X	
Diet	X	

	PREGNANCY	NEWBORN
Family Health History	X	X
Anemia	X	X
Cancer	X	X
Breast Cancer	X	X
Colon Cancer	X	X
Ovarian Cancer	X	X
Other Cancer	X	X
Diabetes	X	X
Heart Disease	X	X
Hypertension	X	X
Lung Disease	X	X
Kidney Disease	X	X
Parathyroid Disease	X	X

8.0 Summary of Implementation Options – Other Items to Track

8.1 Nutrient Intake Guidelines

Daily Required Nutrients
(from all sources)

Vitamin A	2500 IU
Vitamin B1 (Thiamin)	1.4 mg
Vitamin B12	2.6 mcg
Vitamin C	85 mg
Calcium	1000 mg
Vitamin D	***
Vitamin E	22 IU
Folate	600 mcg
Iodine	220 mcg
Iron	27 mg
Liquid	64 oz
Omega 3 (DHA)	300 mg
Probiotics	40 billion CFU multi-strain
Protein	71 g
Zinc	11 mg

*** Take daily amount necessary to achieve 25(OH)D level of 40-60 ng/ml

This checklist is a combination of requirements from institutions including: the U.S. Preventive Services Task Force; the Centers for Disease Control and Prevention; the American College of Obstetricians and Gynecologists; the Institute of Medicine; the National Institute of Child Health and Human Development; and GrassrootsHealth Scientists.

8.2 Lifestyle Guidelines

Lifestyle Recommendations

Alcohol Intake	0/day
Caffeine Intake	200 mg/day max
Fish with high mercury levels	0/day
Fruit / Vegetable pesticides	100% washed
Lead / Radiation exposure	0/day
Physical Exercise (Moderate)	30 min/day
Raw or undercooked meats & seafood	0/day
Smoking	0/day
Toxoplasmosis exposures	0/day
Unpasteurized milk or soft cheeses	0/day

This checklist is a combination of requirements from institutions including: the U.S. Preventive Services Task Force; the Centers for Disease Control and Prevention; the American College of Obstetricians and Gynecologists; the Institute of Medicine; the National Institute of Child Health and Human Development; and GrassrootsHealth Scientists.

9.0 Educational Tools

9.1 CMEs for Medical Personnel

There will be online continuing education courses on Vitamin D, Vitamin D & Pregnancy provided by the vitamin D researchers. They are free to the physicians.

9.2 Scientists Answer Your Questions Webinars

Additional education is offered through scheduled webinars with top vitamin D researchers. Registrants are offered the opportunity to ask their questions of each presenting expert for reliable, evidence-based answers. While no medical advice is offered during these webinars, they do provide an opportunity for participants to obtain a greater understanding of vitamin D and various health concerns for both pregnant and non-pregnant individuals.

The webinars are free for any who wish to participate. Additional information can be found at grassrootshealth.net/webinars.

9.3 Educational Documents & Charts

The following documents and charts can be found at grassrootshealth.net/documentation, downloaded and printed to share:

- Disease Incidence Prevention Chart in ng/ml
- Disease Incidence Prevention Chart in nmol/L
- Scientist's Call to D*action
- Vitamin D FAQs
- Vitamin D FAQs for Pregnancy, Breastfeeding & Babies
- Serum Level vs Intake Charts

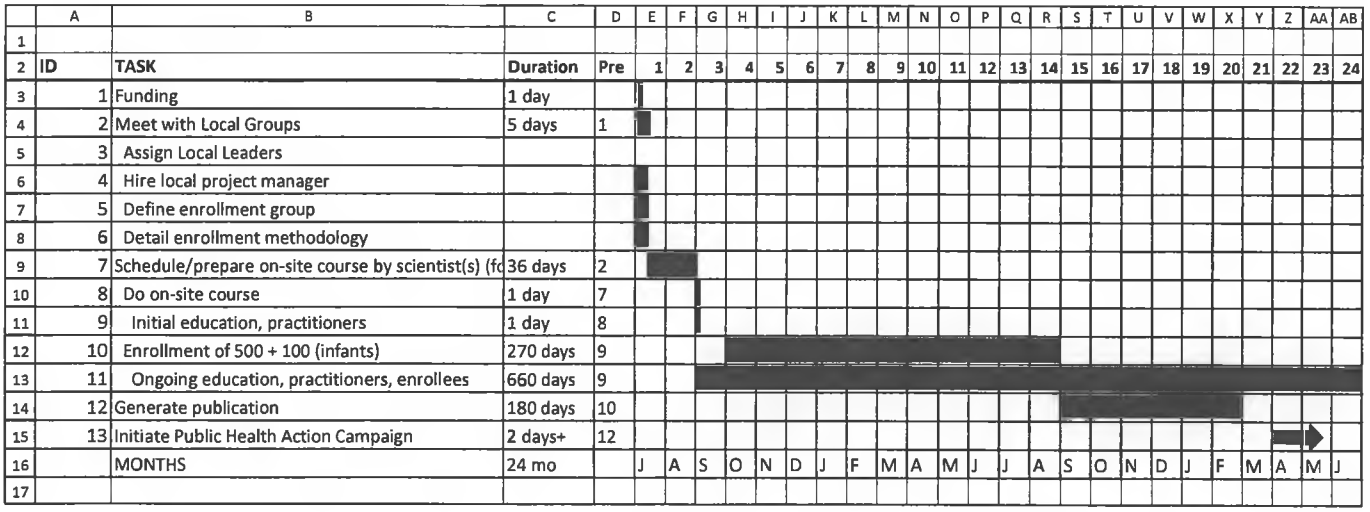
Additional educational handouts on various pregnancy health topics will be accessible to each participant through the MOH system.

9.4 Ongoing Electronic Videos

Videos of interviews with researchers, clinicians will be made available to the project participants throughout the project.

10.0 Cost and Schedule Overview

Protect Our Children NOW! Project	**500 Participants**
Initial Definition Consult	$10,000
Tests, kitting, materials (3)	$52,500
Tests, infants (1)	$17,500
Project Management	$60,000
Education (Online CME)	$35,000
Programming/technical	$35,000
Onsite Support	$90,000
IRB Updates	$10,000
Publication	$50,000
Supplements	$50,000
Travel, Misc.	$50,000
Total	$450,000



ID	TASK	Duration	Pre
1	Funding	1 day	
2	Meet with Local Groups	5 days	1
3	Assign Local Leaders		
4	Hire local project manager		
5	Define enrollment group		
6	Detail enrollment methodology		
7	Schedule/prepare on-site course by scientist(s) (fc	36 days	2
8	Do on-site course	1 day	7
9	Initial education, practitioners	1 day	8
10	Enrollment of 500 + 100 (infants)	270 days	9
11	Ongoing education, practitioners, enrollees	660 days	9
12	Generate publication	180 days	10
13	Initiate Public Health Action Campaign	2 days+	12
	MONTHS	24 mo	

Revised: September 23, 2013

Appendix 1 – Contact Information

Protect Our Children *NOW*! Contact Information

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Appendix 2 – Background Material & References

Studies indicate that serum levels of 25(OH)D in the range of 40-60 ng/ml may (based on the specific disease) reduce or prevent serious health conditions during pregnancy and in the infant.

The National Health and Nutrition Examination Survey (NHANES)[1] reported that 90% of Mexican-American adolescent females had serum 25(OH)D values below 30 ng/mL (the lower end of the normal range proposed by the Endocrine Society); 20% were below 15 ng/mL. For African-American adolescents, the corresponding figures were 99% below 30 ng/mL and 58% below 15 ng/mL. Values for women in their 20s were not appreciably different.

Almost all individuals are unaware of their vitamin D status and many may be deficient. Offering testing will allow individuals to become informed of their vitamin D status. Currently, there is no way to tell a person's serum level without administering a blood test. Even if a person is taking vitamin D supplements, there is a 3 fold variation in the 25(OH)D concentration.

Pregnancy-Related Consequences of Inadequate Vitamin D Status

- Low maternal vitamin D status is associated with increased risk of C-section[2]
- Low vitamin D status in infants at birth is associated with increased risk of RSV infection during the first year of life[3]
- Low maternal vitamin D status is associated with learning impairment in offspring five and 10 years after birth[4]
- Low maternal vitamin D status is associated with increased prematurity, preeclampsia, gestational diabetes, periodontal disease, and TORCH[5–8]
- Low infant vitamin D status in the first year of life is associated with strikingly increased risk of type 1 diabetes prior to age 30[9]
- Low vitamin D status in the first year of life is associated with increased risk of preeclampsia in a female child's own pregnancy when she becomes an adult[10]
- Low maternal vitamin D status is associated with lower birth weight and smaller head circumference[11]

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OBSTETRICS

A randomized trial of vitamin D supplementation in 2 community health center networks in South Carolina

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OBJECTIVE: We sought to determine whether 4000 IU/d (vs 2000 IU/d) of vitamin D during pregnancy is safe and improves maternal/neonatal 25-hydroxyvitamin D [25(OH)D] in a dose-dependent manner.

STUDY DESIGN: A total of 257 pregnant women 12-16 weeks' gestation were enrolled. Randomization to 2000 vs 4000 IU/d followed 1-month run-in at 2000 IU/d. Participants were monitored for hypercalciuria, hypercalcemia, and 25(OH)D status.

RESULTS: Maternal 25(OH)D (n = 161) increased from 22.7 ng/mL (SD 9.7) at baseline to 36.2 ng/mL (SD 15) and 37.9 ng/mL (SD 13.5) in the 2000 and 4000 IU groups, respectively. While maternal 25(OH)D change from baseline did not differ between groups, 25(OH)D monthly increase differed between groups ($P < .01$). No supplementation-related adverse events occurred. Mean cord blood 25(OH)D was 22.1 ± 10.3 ng/mL in 2000 IU and 27.0 ± 13.3 ng/mL in 4000 IU groups ($P = .024$). After controlling for race and study site, preterm birth and labor were inversely associated with predelivery and mean 25(OH)D, but not baseline 25(OH)D.

CONCLUSION: Maternal supplementation with vitamin D 2000 and 4000 IU/d during pregnancy improved maternal/neonatal vitamin D status. Evidence of risk reduction in infection, preterm labor, and preterm birth was suggestive, requiring additional studies powered for these endpoints.

Key words: cholecalciferol, health outcomes, pregnancy, vitamin D

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With avoidance of sunlight exposure due to lifestyle changes, concerns regarding skin cancer, and the resultant widespread use of sunscreen, fewer Americans are meeting their needs for vitamin D.[1-3] A study published in 2002 by the Centers for Disease Control and Prevention and our laboratory at the Medical University of South Carolina (MUSC) revealed that 42% of African American women in their childbearing years exhibited vitamin D deficiency (hypovitaminosis D).[4] More recent publications suggest that the rate of deficiency is higher than previously reported.[2-7]

Until recently, there was no Recommended Dietary Allowance (RDA) for vitamin D, only an adequate intake, which remained at 200 IU/d of vitamin D for decades.[8] A 2010 review of the vitamin D requirements by the Institute of Medicine (IOM) resulted in a revised RDA of 600 IU/d of vitamin D,[9] and suggested that fewer Americans are deficient than previously reported.[9] Using the IOM definition of deficiency of <20 ng/mL (50 nmol/L); however, 2 recent large studies of vitamin D status in pregnant women living at latitude 32°N (South Carolina) showed that African American women were 8 times as likely as Hispanic women, and 20 times as likely as Caucasian women, to have vitamin D deficiency.[2,3,7] Yet little information exists that addresses the vitamin D requirements of the pregnant woman and her fetus.[10]

The recent *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD)-sponsored randomized controlled trial of vitamin D supplementation using 400, 2000, or 4000 IU/d of vitamin D_3 starting at 12 weeks of gestation showed that 400 IU/d was woefully inadequate in achieving vitamin D sufficiency.[7] Optimal 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D$] pro-

From the Division of Neonatology, Department of Pediatrics, Medical University of South Carolina, Charleston (Drs Wagner and Hollis, Ms Bivens, Ms Smith, Ms Murphy, and Ms Shary); the Epidemiologic Research and Information Center, Durham Veterans Affairs Medical Center, Durham (Dr McNeil); and the Department of Obstetrics and Gynecology, Eau Claire Cooperative Health Centers, Columbia (Drs Hamilton, Rodriguez Cook, and Davis, Ms Winkler, and Ms Warner), SC.

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B.W.H. served as a scientific consultant for Diasorin Inc, Stillwater, MN, during the study period. The remaining authors report no potential conflict of interest.

Presented in part at Vitamin D Physiology in Pregnancy: Implication for Preterm Birth and Preeclampsia, Centers for Disease Control and Prevention, Atlanta, GA, April 26-27, 2011, and the annual meeting of the Pediatric Academic Societies, Vancouver, British Columbia, Canada, May 1-4, 2010.

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duction (ie, the point at which its concentration has reached steady-state) was found to occur when total circulating 25-hydroxyvitamin D [25(OH)D] concentration was at least 40 ng/mL (or 100 nmol/L).[7] In addition, only the 4000 IU group achieved optimal status in all women throughout pregnancy, including second trimester, irrespective of race. The translation of vitamin D supplementation at these doses to other women receiving care in a nonuniversity health setting has become essential.

This study was undertaken to shed light on the vitamin D requirements of pregnant women receiving their prenatal care at community health centers. The study's primary hypothesis was that vitamin D supplementation of 2000 or 4000 IU/d during pregnancy was safe and effective in achieving vitamin D sufficiency and would result in improved maternal and neonatal health status. It was further hypothesized that the 4000 IU dose would result in a greater increase in 25(OH)D than the 2000 IU dose. The coprimary outcome measures were the change in circulating 25(OH)D concentration from baseline to the completion of pregnancy in the mother, and her neonate's 25(OH)D concentration at birth.

Materials and Methods

Study design

This was a 2-center, randomized, double-blinded study of vitamin D supplementation (Food and Drug Administration Investigational New Drug #66,346; ClinicalTrials.gov #NCT00412087). The study was approved by the MUSC Institutional Review Board (IRB) for Human Research (HR# 16476) and the Palmetto Baptist Hospital (Columbia, SC) IRB for Human Research (PH IRB# 2007-25). Written informed consent was obtained from each participant. Women <16 weeks' gestation were eligible for participation in the study.

A precedent NICHD vitamin D supplementation trial during pregnancy had begun in 2004 with baseline 25(OH)D analysis revealing marked deficiency among darker pigmented women.[3] Further, because 400 IU/d of vitamin D had already been shown to be ineffective in maintaining adequate vitamin D status during pregnancy[7,11-14] and because the majority of women being recruited in this study were either African American or Hispanic, with darker pigmentation and a greater likelihood of vitamin D deficiency, a control group that would receive 400 IU/d of vitamin D was considered unethical by the scientific review committee as well as the research team. Hence, a control arm was not included a priori in the study design.

Study setting

This study was conducted from Nov. 21, 2006, through June 30, 2010, at Eau Claire Cooperative Health Center (ECCHC) in Columbia, SC, and Northwoods Community Health Center (NCHC) in North Charleston, SC. ECCHC and NCHC are both Section 330 community health centers. At least 60% of the ECCHC and NCHC patient populations are of racial/ethnic minority groups, including African American and Hispanic women. The populations being served by ECCHC and NCHC are among the poorest in South Carolina; the majority of women are 100% below the federally designated poverty level and many women rank 200% below federal poverty level.

Inclusion and exclusion criteria

The inclusion criteria were: maternal age ≥16 years; confirmed singleton pregnancy of <16 completed weeks of gestation at the time of enrollment; and intention to receive ongoing prenatal care at the community health center where consent was obtained.

Mothers with preexisting calcium or parathyroid conditions or who required chronic diuretic or cardiac medication therapy, including calcium channel blockers, were not eligible for enrollment into the study. Mothers with active thyroid disease (eg, Graves, Hashimoto, or thyroiditis) also were not eligible to participate in the study; however, mothers on thyroid supplement with normal serological parameters could participate in the study if they were without any other endocrine dysfunction.

Randomization and intervention

Upon enrollment into the study, expectant mothers' vitamin D status was assessed by measuring total circulating 25(OH)D and parathyroid hormone (PTH). Based on this initial 25(OH)D level, the randomization to 2000 or 4000 IU/d of vitamin D_3 was stratified using a cut point of 32 ng/mL. Randomization lists were generated by computer prior to the start of the study. Randomization assignment was blinded to all participants and to the investigators except for the study biostatistician. Dose groups were identified for logistical purposes using 6 letters (3 per dose group) as an additional measure against inadvertent unblinding.

Adherence to medication regimen

Adherence to the vitamin D supplementation regimen was measured by maternal self-report and pill counts at each follow-up visit.[15] If a woman missed 1 prenatal visit, her next month's supply of vitamins was mailed to her or delivered to her residence. If a woman had >2 missed visits or if she failed to take at least 50% of the prescribed vitamin D pills, she was exited from the study.

Study protocol

Gestational age at enrollment

Gestational age was based on last menstrual period. If a woman was unsure of this date, the obstetrical estimate at the time of the visit was used. If, at the 20-week fetal ultrasound, it was determined by the obstetrician that the gestational age was incorrect, the revised gestational age was used.

Initial study visit

Baseline blood and urine samples were obtained following each participant's consent at the initial visit (10 to <16 weeks). Irrespective of enrollment gestational age, vitamin D supplementation did not begin before the 12th week of gestation (12 and 0/7 weeks).

Subsequent study visits

Participants were followed with monthly study visits, which continued until delivery. These visits coincided with routine obstetrical visits. There was 1 additional visit with mother and infant 2 weeks' postpartum.

Completion of questionnaires

Participants completed questionnaires used in the NICHD vitamin D preg-

nancy trial,[7] which included sociodemographic information, baseline health status, and medical history at the first visit. At the second visit, the Block Food Frequency Questionnaire (Block, Berkeley, CA) was completed to ascertain generalized eating pattern, with specific calculation of calcium and vitamin D intake.[16-21] An interim maternal health history questionnaire also was completed at each visit with the assistance of the study coordinator to ascertain adverse events, and type and frequency of acute illnesses such as respiratory, gastrointestinal, and other viral and/or bacterial illnesses. A review of medications and doctor's visits was obtained at that time. After delivery, the newborn record of each infant was reviewed for mode of delivery, birthweight (grams), and gestational age.

Blood and urine samples

Maternal blood samples were collected at the first visit, then every other obstetrical visit and at the time of delivery. Maternal urine samples were collected at each visit. Cord blood was obtained at delivery. If the cord blood sample could not be obtained, a neonatal blood sample was drawn within 2 weeks of delivery.

Materials

Source of vitamin D

Vitamin D tablets (1600 and 3600 IU) were manufactured by Tishcon Corp (Westbury, NY) a Good-Manufacturing-Practice facility. Hoffman-La Roche Ltd (Basel, Switzerland) supplied the cholecalciferol content contained in the vitamin D tablet manufactured by Tishcon Corp. The tablet vitamin concentration was verified by the company every 6 months and by an independent laboratory chosen by the investigators (Heartland Assays, Ames, IA) using high-performance liquid chromatograph with ultraviolet light detection to ensure the tablets met label claim throughout the study; these results were reported to the MUSC Investigational Drug Department. Tablets were maintained in the MUSC Investigational Drug Division of Pharmacy until the time that they were dispensed to enrolled subjects at ECCHC or NCHC.

Source of prenatal vitamins

Prenatal vitamins (400 IU vitamin D_3/tablet) prescribed at study entry were Myadec multivitamin-multimineral supplement (distributed by Pfizer Consumer Healthcare, Morris Plains, NJ). Those mothers unable to swallow a prenatal vitamin were given a Flintstones Complete chewable vitamin (Bayer Healthcare, Morristown, NJ) (400 IU vitamin D_3 per vitamin).

Study measures

Maternal sociodemographic questionnaire

Upon enrollment in the study, each mother was asked to complete a sociodemographic questionnaire to ascertain maternal age, race, educational level, occupation, and insurance status.

Race/ethnicity definition

Each mother was asked to describe the racial/ethnic group to which she belonged, by selecting any applicable categories from African American, Caucasian, Hispanic, American Indian, Asian, and other.

Pregnancy intake and surveillance survey

Upon enrollment, each woman was asked to complete a health assessment questionnaire to ascertain her use of medications (checklist) and over-the-counter preparations that may have influenced vitamin D/calcium homeostasis. Additional questions concerned use of cigarettes and alcohol, and overall health status.

Pregnancy health status, and labor and delivery characteristics and complications

Characteristics of each mother's health status and complications during pregnancy, labor, and delivery were recorded. Complications at the time of delivery were listed according to American Congress of Obstetricians and Gynecologists definitions. In addition, if the mother required hospitalization, a copy of the hospital record was obtained after she signed a release of medical information form. Any acute illnesses or development of pregnancy-related conditions that were not preexisting also were recorded. When appropriate, the Data Safety and Monitoring Committee (DSMC) and the IRB were notified of any adverse events.

Season

The season that each blood sample was drawn was defined as spring (April through May), summer (June through September), fall (October through November), and winter (December through March).

Maternal body mass index measurement

Prepregnancy height and weight of each mother were recorded at the first outpatient visit to determine body mass index (weight [kg]/height [m^2]). During subsequent visits, only the mother's weight was recorded, and the initial height and updated weight were used to calculate body mass index at each outpatient visit.

Neonatal growth parameters

At the postpartum visit, the infant's weight in grams, head circumference in centimeters, and length in centimeters were recorded. The growth parameters were then plotted using Fenton growth curves, which facilitate the calculation of z-scores and permit the more precise assessment of growth of infants who are born preterm.[22]

Laboratory measurements

Maternal and cord blood/neonatal total circulating 25(OH)D assays

A rapid, direct radioimmunoassay developed in an author laboratory (B.W.H.) and manufactured by Diasorin Corp (Stillwater, MN) was used to measure total circulating 25(OH)D concentration in serum samples as previously described.[7,23] Based on clinical laboratory classifications,[24,25] a priori, deficiency was defined as total circulating 25(OH)D 20 ng/mL (50 nmol/L), insufficiency as ≥20-32 ng/mL (≥50-80 nmol/L), and sufficiency as ≥80 nmol/L (≥32 ng/mL).[10,25-28] The interassay and intra-assay coefficient of variation was ≤10%.

Maternal and infant concentrations of serum calcium, creatinine, and phosphorus

Maternal serum total calcium, creatinine, and inorganic phosphorus were measured bimonthly (maternal) and at delivery (cord blood) by MUSC Clinical Chemistry Laboratory using standard methodology and laboratory normative data.

Monthly maternal urinary calcium:creatinine ratio

Urinary calcium and creatinine were measured monthly for each mother by the MUSC Clinical Chemistry Laboratory (to convert mg/dL of calcium to mmol/L, multiply the value by 0.25. To convert mg/dL of creatinine to mmol/L, multiply by 0.088), then expressed as a ratio (urinary calcium [mg/dL]: creatinine [mg/dL] ratio).

Maternal and cord blood concentrations of circulating intact PTH

Intact PTH (iPTH) was measured in serum samples by immunoradiometric assay as previously described.[7] The adult normal range for iPTH in our laboratory is 1.3-5.4 pmol/L. Higher vitamin D levels are associated with lower iPTH; as vitamin D status improves, iPTH declines.[29]

Safety monitoring

All study participants were monitored monthly for hypervitaminosis D. The first sign of hypervitaminosis D is hypercalciuria, of which, urinary calcium:creatinine ratio is the most sensitive indicator. Operationally, we defined a priori caution limits for hypervitaminosis D as a urinary calcium:creatinine (mg/dL) ratio $\geq$0.8. The study's DSMC reviewed the quarterly summary reports that were generated for all subjects enrolled in the study. Whenever any patient was to exceed the caution limit, a specific case study was to be initiated to examine the contribution of confounding factors (eg, diet, sunlight exposure). Operationally, we were to stop vitamin D_3 supplementation if the urinary calcium:creatinine ratio (measured monthly) exceeded 1.0 or if the circulating 25(OH)D level (measured bimonthly) exceeded 100 ng/mL, and the DSMC and IRB were notified immediately. The principal investigator of the study reviewed all laboratory results on a weekly basis to identify potentially abnormal values.

Statistical methods

Sample size and power considerations

A total of 148 participants were to be enrolled in the study with 74 per supplementation arm. For one primary endpoint of change in 25(OH)D level between baseline and final measurements, this sample size would support the detection of a 10-ng/mL difference between dose groups with 80% power using a 2-sided t test at $\alpha = 0.05$. This calculation assumed that the SD of 25(OH)D measurements at a single time point was approximately 10, that there would be a low correlation ($\rho = 0.25$) between the baseline and final measurements, and that a substantial proportion (up to 50%) of participants may be lost to follow-up. This calculation was robust to changes in the assumption regarding the magnitude of correlation between measurements made over time; if a higher correlation were to be present, the study's power would be increased.

Statistical analyses

Primary analysis

The a priori primary data analysis focused on comparisons of change in serum 25(OH)D between the 2 dose groups. While participants were randomized within each stratum, as described above, the strata were extremely imbalanced at the close of the study due to the infrequent occurrence of 25(OH)D levels >32 ng/mL (Figure 1). Thus, the primary comparison for planning purposes was a 2-sample test of dose group differences in the change in 25(OH)D levels, regardless of stratum. This comparison also was performed using the Wilcoxon rank sum test as a sensitivity analysis to the normality assumption. The latter test is reported as the primary analysis. Statistical significance is claimed for $P < .05$. Due to the multisite implementation of the study, we also report results controlled for site using multivariable models.

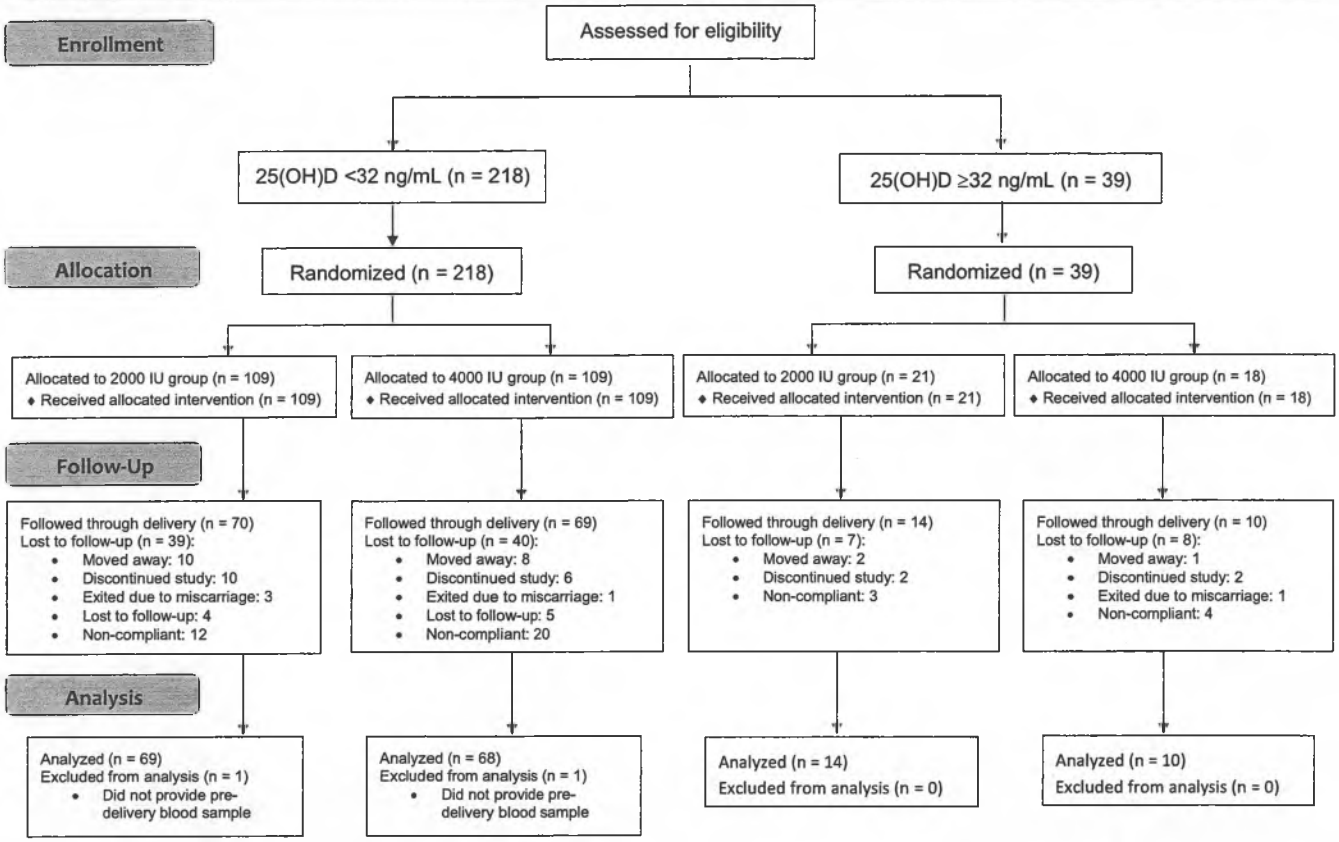
Secondary analysis

In secondary analyses, multilevel mixed-effects models were used to estimate the average monthly rate of change in 25(OH)D, compare this rate between dose groups, and explore the effects of covariates on the rate of change.[30] These models included fixed effects for dose group, time, and the group-time interaction, and a random intercept effect, with additional covariate effects as required. Time was considered a continuous variable, measured in months rather than assuming structured visit occurrences. An unstructured covariance matrix was assumed. The same approach was used to evaluate the longitudinal association between 25(OH)D and calcium, iPTH (log transformed), phosphorus, and urinary calcium, creatinine, and calcium:creatinine levels. The cumulative occurrence of pregnancy complications was compared between dose group levels using logistic regression. All analyses were performed using software (SAS, version 9.3; SAS Institute Inc, Cary, NC).

Participant attrition and missing data

Because the primary endpoint was change in 25(OH)D from baseline to delivery, the primary analysis was restricted to participants who remained in the study until delivery and provided a blood sample within 6 weeks prior to delivery, at delivery, or at the postdelivery visit (completers-only analysis). Typically, multiple imputation would be used to impute missing values in support of the favored intention-to-treat analytic approach. Because the multiple imputation model for this analysis would have required variables also measured in the final blood sample, however, it could not be used to impute cases with a missing final blood sample. Thus, to assess the primary findings' robustness to assumptions about the missing data, we performed a sensitivity analysis under the following assumptions: cases with missing endpoints experienced no change in both groups; experienced the group-specific median change observed in com-

FIGURE 1
CONSORT flow diagram for this randomized clinical trial



CONSORT, consolidated standards of reporting trials; *25(OH)D*, 25-hydroxyvitamin D.

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pleters; experienced no change in the 2000 IU group and minimal change in the 4000 IU group. In the secondary analyses using multilevel mixed-effects models for longitudinal modeling, all available data points were used, as it is not necessary to delete cases with missed time points when using this approach.

RESULTS

Baseline characteristics

A total of 257 women consented to participate in this study. Of those, 161 (63%) provided complete data regarding the primary endpoint (Figure 1). The sociodemographic characteristics of the active cohort are found in Table 1. After controlling for race and study site, no characteristics differed significantly between groups.

The mean maternal baseline 25(OH)D was 22.7 ng/mL (SD 9.7); this did not differ significantly between dose groups (direct comparison $P = .43$; controlled for study site $P = .43$; controlled for race $P = .51$). Estimated mean baseline serum iPTH was 16.4 pg/mL (SD 6.9) and mean serum calcium was 9.3 mg/dL (SD 0.3). The iPTH and calcium differed minimally between dose groups, with participants randomized to the 4000 IU group being 0.2 pg/mL lower in PTH ($P = .76$) and 0.1 mg/dL lower in calcium ($P = .74$) than those randomized to the 2000 IU group.

There were significant differences in baseline 25(OH)D according to race: African American participants had the lowest values, with an estimated mean of 18.5 ng/mL (SD 8.4), while Hispanic and Caucasian participants had notably higher mean values of 26.1 ng/mL (SD 7.5) and 29.5 ng/mL (SD 14.4), respectively (P value for overall comparison $< .001$). Baseline iPTH and calcium values did not differ significantly by race (iPTH, $P = .11$; calcium, $P = .40$).

Primary analysis

Primary endpoint

The overall maternal 25(OH)D change from baseline was estimated to be +14.1 ng/mL (SD 12.7) ($P < .001$). The mean change from baseline for the 2000 and 4000 IU groups was +12.9 ng/mL (SD 12.8) and +15.4 ng/mL (SD 12.6), respectively (group comparison of change $P = .23$; $P = .40$ adjusted for race and study site). At the last study visit prior to delivery, the average 25(OH)D levels were 36.2 ng/mL (SD 15.0) in the 2000 IU group and 37.9 ng/mL (SD 13.5) in the 4000 IU group. The difference between the 2 groups at this time point was not statistically significant in direct comparisons ($P = .29$) or after controlling for race and study site ($P = .47$). Overall, 31 of 83 (37.4%) of the 2000 IU group, and 36 of 78 (46.2%) of the 4000 IU group achieved 40 ng/mL at this time point ($P = .27$), the level at which con-

TABLE 1
Sociodemographic and clinical characteristics by self-reported race/ethnicity

Characteristic	Total cohort N = 257	2000 IU/d n = 130	4000 IU/d n = 127	*P* value	*P* value controlled for race and site
Maternal age (y), mean ± SD	25.0 ± 5.1	24.5 ± 5.3	25.4 ± 5.0	.076	.116
Gestational age at enrollment (wk), mean ± SD	12.4 ± 1.8	12.4 ± 1.7	12.4 ± 2.0	.48	.60
Planned pregnancy	95 (37.0%)	47 (36.2%)	48 (37.8%)	.79	.64
Gravidity	1 (0–7)	1 (0–7)	1 (0–7)	.37	.29
Primigravida	97 (37.7%)	51 (39.2%)	46 (36.2%)	.62	.55
Parity	1 (0–4)	0 (0–4)	1 (0–4)	.069	.071
BMI ≥30	70 (27.2%)	32 (24.6%)	38 (29.9%)	.34	.34
Race					
African American	124 (48.3%)	61 (46.9%)	63 (49.6%)	.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					
<High school	72 (28.0%)	35 (26.9%)	37 (29.1%)	.85	.74
High school	104 (40.5%)	56 (43.1%)	48 (37.8%)		
Some college	57 (22.2%)	27 (20.8%)	30 (23.6%)		
≥Associates degree	24 (9.3%)	12 (9.2%)	12 (9.5%)		
Employed	116 (45.1%)	60 (46.2%)	56 (44.1%)	.74	.61
Season (April through September)	163 (63.4%)	83 (63.9%)	80 (63.0%)	.89	.83
Insurance status					
None	93 (36.2%)	48 (36.9%)	45 (35.4%)	.96	.81
Medicaid	117 (45.5%)	59 (45.4%)	58 (45.7%)		
Private	47 (18.3%)	23 (17.7%)	24 (18.9%)		
Obstetrical history					
Preterm birth	12 (4.7%)	5 (3.9%)	7 (5.5%)	.57	—
Preeclampsia	11 (4.3%)	5 (3.9%)	6 (4.7%)	.77	—
Gestational diabetes	7 (2.7%)	4 (3.1%)	3 (2.4%)	1.00	—
Diabetes (type 1 or 2)	5 (2.0%)	2 (1.5%)	3 (2.4%)	.68	—
Chronic hypertension	2 (0.8%)	0	2 (1.6%)	.24	—
Subjective health rating	9 (3–10)	9 (3–10)	10 (5–10)	.29	.18

Continuous and ordinal variables reported as median (range) unless otherwise noted, and compared between groups using Wilcoxon rank sum test. Categorical variables reported as frequency (%), and compared between groups using χ^2 test or Fisher exact test. *P* values controlled for race and site were obtained using multivariable linear or logistic regression, when sample size permitted. Missing data as follows: gestational age, n = 3.
BMI, body mass index.

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version of 25(OH)D to $1,25(OH)_2D$ is optimized during pregnancy.[7]

Neonatal 25(OH)D as measured in cord blood

The mean cord blood 25(OH)D level, as measured on 144 infants, was 0.7 (SD 0.3) times that of maternal predelivery 25(OH)D. Overall, the mean cord blood 25(OH)D level was 24.5 ng/mL (SD 12.0): 22.1 ng/mL (SD 10.3) in the 2000 IU group and 27.0 ng/mL (SD 13.3) in the 4000 IU group ($P = .024$). The correlation between the infants' and mothers' 25(OH)D levels was estimated to be $r = 0.68$ ($P < .001$).

PTH and calcium

Predelivery calcium values did not differ significantly between dose groups: the estimated mean values were 8.9 mg/dL (SD 0.4) and 9.0 mg/dL (SD 0.4) in the 2000 and 4000 IU groups, respectively ($P = .17$). The estimated change from baseline was −0.3 mg/dL in the 2000 IU

group and −0.2 mg/dL in the 4000 IU group ($P = .22$, analysis of covariance). The significance of these results did not change after controlling for race and study site. Predelivery iPTH values differed significantly between dose groups, with estimated mean values of 17.5 pg/mL (SD 8.2) and 15.2 pg/mL (SD 9.3) in the 2000 and 4000 IU groups ($P = .023$). This difference remained significant ($P = .044$) after controlling for race. The dose groups also differed slightly in their PTH change from baseline. Specifically, participants randomized to the 2000 IU/d group had a mean increase in PTH of 0.9 pg/mL (SD 8.0), while those randomized to the 4000 IU/d group had a mean decline in PTH of 1.1 pg/mL (SD 9.0) ($P = .034$, analysis of covariance). This finding became marginally significant after controlling for race and site ($P = .054$).

Complications of pregnancy

Table 2 describes the frequency of common complications of pregnancy and compares them between randomization groups. Among the 161 participants who provided a primary endpoint measure, the most common complication was infection, experienced by 74 women (46.0%). The other complications occurring in >10% of women were preterm labor (37/161, 23.0%), gestational diabetes (18/161, 11.2%), and preterm delivery (19/161, 11.8%). A total of 50 participants had either preterm labor or preterm delivery (31.1%). The remaining conditions occurred in <3% of the participants. The frequency of occurrence did not differ significantly between randomization groups for any of the conditions considered; however, the frequency of preterm labor was marginally significantly different between groups ($P = .091$) and became more significant ($P = .060$) after controlling for the effects of race. The total number of complications differed significantly between groups ($P = .044$); this comparison remained marginally significant ($P = .061$) when controlling for race.

Neonatal growth parameters

Figure 2 illustrates the distribution of neonatal weight and head circumference across the major Fenton percentiles for each dose group. There was an association between percentile and dose group for neonatal weight ($P = .018$, proportional odds model), but not head circumference ($P = .79$, proportional odds model). Specifically, the 4000 IU group participants had 2.40 (95% confidence interval, 1.26–4.61) times the odds of having an infant in the 50th percentile, compared to the 2000 IU group. This effect remained significant after controlling for the effects of race and study site ($P = .006$).

TABLE 2
Clinical conditions reported during pregnancy among trial completers (n = 161)

Medical condition	Total cohort N = 161	2000 IU/d n = 83	4000 IU/d n = 78	*P* value
Preterm labor[a]	37 (23.0%)	24 (28.9%)	13 (16.7%)	.091
Preterm delivery[b] (<37 0/7 wk)	19 (11.8%)	10 (12.1%)	9 (11.5%)	1.00
Preterm labor or delivery[a,b]	50 (31.1%)	29 (34.9%)	21 (26.9%)	.31
Hypertension[c]	4 (2.5%)	3 (3.6%)	1 (1.3%)	.62
Infection[d]	74 (46.0%)	41 (49.4%)	33 (42.3%)	.43
Gestational diabetes[e]	18 (11.2%)	11 (13.3%)	7 (9.0%)	.46
Any above comorbidity	107 (66.5%)	58 (54.2%)	49 (45.8%)	.40
Total comorbidity count	.044			
None	54	25 (30.1%)	29 (37.2%)	
1	61	31 (37.4%)	30 (38.5%)	
2	21	8 (9.6%)	13 (16.7%)	
3	20	15 (18.1%)	5 (6.4%)	
4	5	4 (4.8%)	1 (1.3%)	
Nonrepeat cesarean[f]	58 (36.0%)	31 (53.5%)	27 (46.6%)	.61

P values for comparison of 2000 vs 4000 IU/d were obtained using Fisher exact test, except for analysis of total comorbidity count, which used Poisson regression. Missing data as follows: nonrepeat cesarean, n = 13.

[a] As documented in medical record that included hospitalization >24 h; [b] As documented in medical record; [c] As documented in medical record that included gestational hypertension, preeclampsia, HELLP syndrome, worsening hypertension in women with existing hypertension; [d] New infection documented in medical record that included urinary tract infections, bacterial vaginosis, flu or flu-like illness, or any illness that required antibiotic or antifungal therapy (did not include preexisting herpes simplex infections for which antiviral therapy was prescribed); [e] As documented in medical record following glucose tolerance test (did not include those with preexisting diabetes type 1 or 2); [f] Mode of delivery by cesarean section that was not repeat cesarean or vaginal delivery.

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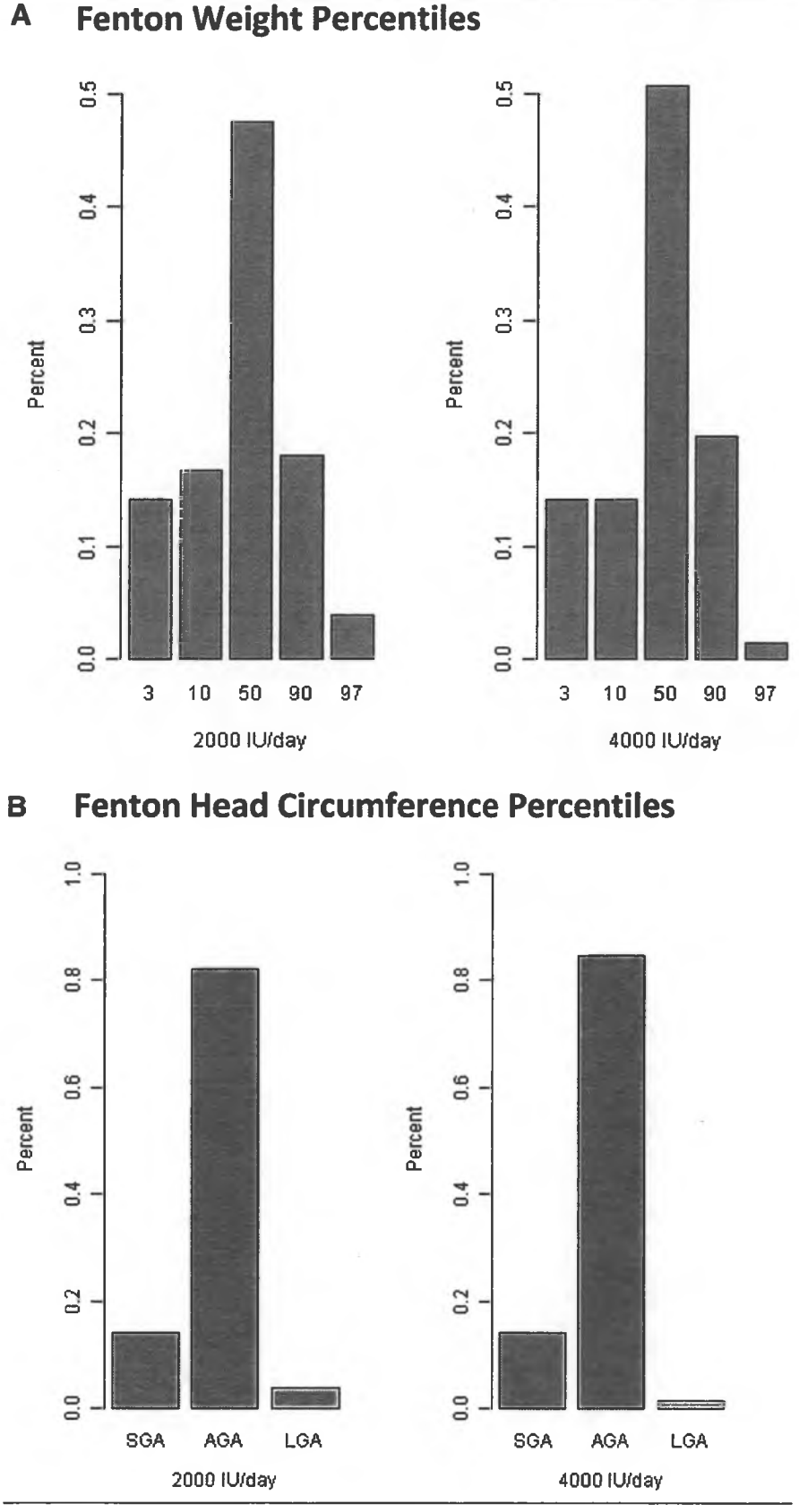
Safety parameters

Neither dose group experienced any instances of hypercalciuria or hypercalcemia. In longitudinal models assessing the relationship of 25(OH)D with serum calcium and phosphorus and urinary calcium, creatinine, and the calcium:creatinine ratio, statistically significant associations were observed with urinary calcium and urinary creatinine, after adjustment for race and study site. Specifically, we estimate that urinary calcium decreased 3.5 mg/dL per 10 ng/mL increase in 25(OH)D ($P = .02$). Urinary creatinine decreased an estimated 16.5 mg/dL per 10 ng/mL increase in 25(OH)D ($P = .004$). Figures 3 and 4 illustrate the time courses of serum calcium and the urinary calcium:creatinine ratios.

Sensitivity analysis

A sensitivity analysis was performed to determine the robustness of the primary findings to assumptions about the missing data. When all participants with missing final measurements were assumed to have experienced no change from baseline, the significance of our findings was unchanged in direction ($P = .69$). However, when we assumed no change among those with missing end-

FIGURE 2
Infants by treatment group plotted by Fenton growth curve percentiles



points in the 2000 IU group, but even a minimal 0.1-ng/mL change among those with missing endpoints in the 4000 IU group, the group comparison became statistically significant at $P \leq .025$. Our most extreme imputation of the observed group medians, assuming that those with no final measurement were indistinguishable from the other members of their dose group, resulted in a highly significant group comparison at the $P = .0006$ level.

Secondary analyses

Serum 25(OH)D

Based on 1042 longitudinal measurements contributed by 257 participants, the monthly change in 25(OH)D level (Figure 5) was estimated to have a significant quadratic component ($P < .0001$). This effect differed significantly between dose groups ($P < .01$), such that estimated 25(OH)D levels of participants randomized to the 2000 IU group increased more slowly than the levels of those randomized to the 4000 IU group.

In an expanded model, race was a significant predictor of baseline status ($P < .0001$) and 25(OH)D change over time ($P < .001$), but not group differences in change over time. Baseline 25(OH)D level did not influence the change in 25(OH)D over time in a clinically significant manner. In the final longitudinal model of 25(OH)D, baseline values were estimated to be 19.2, 26.8, and 30.1 ng/mL among African American, Hispanic, and Caucasian participants, respectively, assigned to the 2000 IU dose group. The 4000 IU dose group was estimated to have baseline values 0.11 ng/mL lower than the 2000 IU group ($P = .94$). Model-based estimates of 25(OH)D levels over time are provided in Table 3 for each race-by-dose sub-

Percent of infants within each treatment group who were at 3rd, 10th, 50th, 90th, and 97th percentiles for **A**, weight and **B**, head circumference. Less than 10th percentile is defined as small for gestational age (SGA); 10th-90th percentile as appropriate for gestational age (AGA); and >90th percentile as large for gestational age (LGA).

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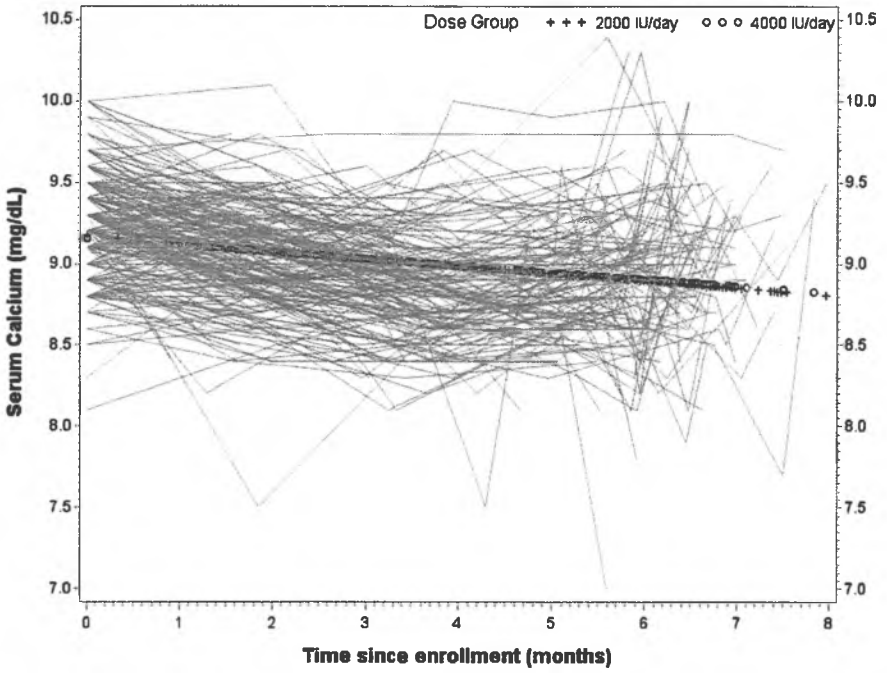
group, assuming a 6-month supplementation period.

Complications of pregnancy

Baseline 25(OH)D level was not predictive of complication occurrence except for preterm labor (odds ratio [OR], 0.61 per 10 ng/mL; $P = .033$) and combined preterm labor or delivery (OR, 0.56 per 10 ng/mL; $P = .007$), which became nonsignificant after controlling for race and study site (labor alone OR, 0.78 per 10 ng/mL; $P = .31$; and labor or delivery OR, 0.64; $P = .052$). Predelivery 25(OH)D was significantly predictive of preterm delivery (OR, 0.56 per 10 ng/mL; $P = .004$) and combined preterm labor or delivery (OR, 0.73 per 10 ng/mL; $P = .015$), which remained significant (delivery alone OR, 0.51 per 10 ng/mL; $P = .002$; and labor or delivery OR, 0.73 per 10 ng/mL; $P = .018$) after controlling for the effects of race and study site. Additional analyses by mean 25(OH)D (from third visit onward) indicated negative associations with preterm delivery (OR, 0.46 per 10 ng/mL; $P = .001$) and infection (OR, 0.74 per 10 ng/mL; $P = .026$) after controlling for race and study site.

FIGURE 3
Longitudinal maternal serum calcium (mg/dL) by treatment group



Longitudinal serum calcium (mg/dL) with group overlay by treatment group, with each participant's serum calcium values plotted over time.

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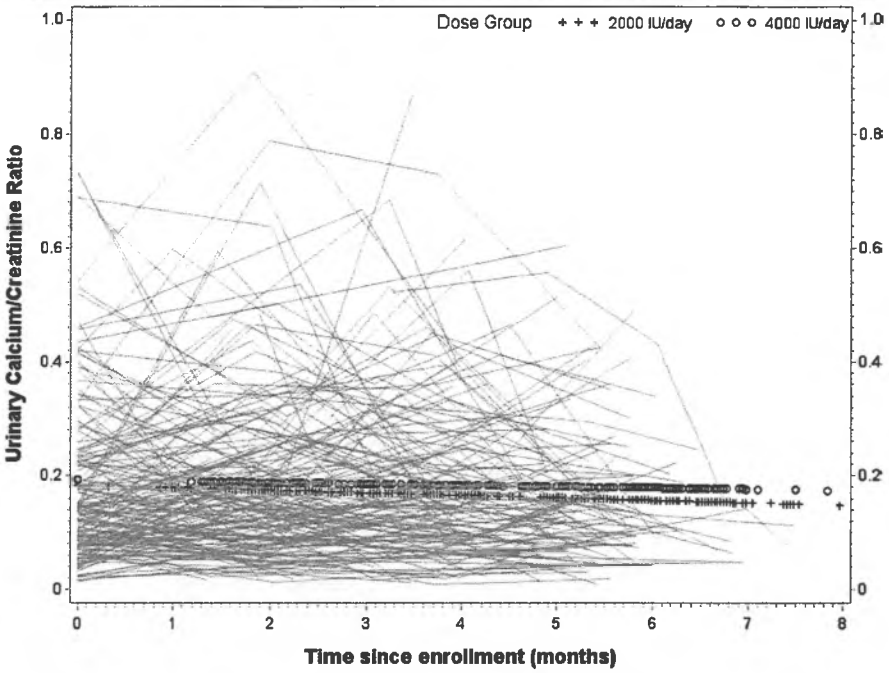
Comment

In this randomized trial of vitamin D supplementation in a diverse group of women receiving prenatal care at 2 community health center networks in South Carolina, women receiving daily doses of 2000 and 4000 IU did not differ in their predelivery vitamin D status; however, 4000 IU/d was superior to 2000 IU/d in raising maternal vitamin D status over time and in achieving neonatal vitamin D sufficiency, and was associated with lower maternal iPTH. While caution must be given when interpreting these findings, as the study was not designed as an equivalence study, but rather, to demonstrate superiority of one dose over the other, secondary analyses did show a dose-dependent effect with respect to preterm labor, preterm birth, and risk of infection.

Although it was expected that both the 2000 and 4000 IU/d of vitamin D groups would experience an increase in circulating 25(OH)D levels, with the greatest increase in those women in the 4000 IU group, there was concern that the 4000 IU group would have greater risk of hypervitaminosis D, which did not occur. In neither the 2000 IU nor the 4000 IU arms of the study were there any episodes of hypercalciuria or hypercalcemia. Urinary calcium:creatinine ratios and serum calcium, phosphorus, and creatinine levels were comparable in both groups and were well within normal limits for adults, including pregnant women. This is similar to our findings in the NICHD supplementation trial.[7]

Based on national and regional data, it is clear that vitamin D deficiency is an emerging health issue that affects all ethnic and racial groups in the United States, but more significantly those with darker pigmentation such as African Americans and Hispanics.[2-4,7,31] The pregnant woman and her unborn fetus represent an important and often neglected segment of the US population.[26] It is during pregnancy that fetal cell signaling patterns are set into motion, affecting lifelong health status.[32-34] Most recently, the link between vitamin D deficiency and extraskeletal systems has been suggested: vitamin D deficiency is associated with acute and chronic infections,[35-40] and later, lifelong sequelae, with notably increased risk of autoimmune diseases such as rheumatoid arthritis,[41] systemic lupus erythematosus,[42] multiple sclerosis,[43,44] type 1 diabetes,[45,46] and certain cancers.[45,47-52] Specific to the pregnant woman and her fetus, epidemiological studies report an association between vitamin D deficiency and an increased risk of hypertensive disorders of pregnancy, including preeclampsia,[53-62] preterm birth,[56] and mode of delivery.[63] Additionally, chronic vitamin D deprivation in the mother and fetus appears to have lasting effects on skeletal integrity[26,64-66] and fetal growth.[67] These findings are supported by the earlier NICHD study[7,68] and by this study, where secondary analyses found a suggestive pattern of associations between total circulating 25(OH)D and preterm labor, preterm birth, and infection, even after controlling for race. In

FIGURE 4
Longitudinal maternal urinary calcium:creatinine ratios by treatment group



Longitudinal urinary calcium:creatinine ratios (mg/dL) with group overlay by treatment group, with each participant's urinary calcium:creatinine ratios plotted over time.

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addition, neonates in the 4000 IU group were more likely to be average for gestational age by weight than those in the 2000 IU group, which may reflect maternal glucose homeostasis, as larger babies are traditionally associated with gestational diabetes.[69] We note, however, that the maternal associations were identified in secondary analyses, as the study was not powered to detect changes in the rate of complications as primary endpoints.

There were certain limitations in this study, the most notable being the nonadherence to protocol of some of the subjects. Women who were nonadherent would have a lower rise in their circulating 25(OH)D compared to women who were adherent. Thus, the treatment effect was reduced as a whole. The decline in 25(OH)D during the last trimester, possibly due to increased conversion to $1,25(OH)_2D$, may also reflect the cumulative dropout of subjects during the course of pregnancy.

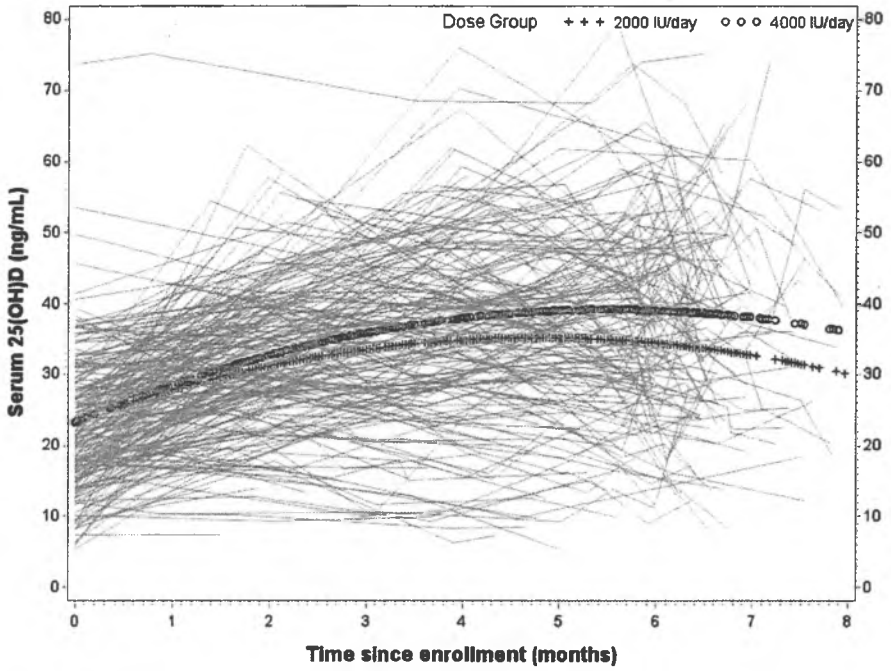
In evaluating our findings in the context of earlier studies, it is interesting to note that Mallet et al[70] reported vitamin D supplementation of 1000 IU/d during the last trimester of pregnancy resulted in only a 5- to 6-ng/mL increase in circulating 25(OH)D levels in maternal and cord serum. Similarly, Datta et al,[71] in their study of 160 pregnant minority women in England who were provided with 800-1600 IU vitamin D for the duration of their pregnancy, found that circulating 25(OH)D levels increased from 5.8 ng/mL (SD 0.9) at the beginning of pregnancy to 11.2 ng/mL (SD 6.3) at term. The Vieth et al[72] and Heaney et al[73,74] data, and our own data with pregnant and lactating women,[7,28,68,75] have shown that doses exceeding 1000 IU/d of vitamin D (2000-10,000 IU/d) are required to achieve a robust nutritional vitamin D status. Further, on a per-kilogram basis, 400 IU has been shown to be adequate to achieve normal vitamin D status in neonates and infants; however, for a pregnant woman weighing on average 20 times that of a newborn infant receiving the same dose of vitamin D–namely 400 IU/d–that dose is irrelevant.[7,75,76]

The nutritional vitamin D status of the neonate is completely dependent on the vitamin D stores of the mother.[78-82] This premise was reconfirmed by this study. Thus, if the mother is hypovitaminotic D, her infant will show depleted vitamin D stores. The effects of acute vitamin D deprivation are known to result in rickets in the rapidly growing child and osteopenia and osteoporosis in the mother. This result is especially prevalent in darker pigmented individuals, as reflected in a steady and significant rise in nutritional rickets in breast-fed infants, mainly in the African American population.[78-82] As was shown by this study and an earlier study, substantially improving the nutritional vitamin D status of the neonate at birth through higher-dose maternal supplementation of vitamin D during pregnancy–above the suggested 400 or 600 IU/d–to achieve maternal sufficiency should alleviate this problem. Thus, we strongly believe that the current RDA for vitamin D in all age groups, but particularly pregnant women with darker pigmentation, is inadequate.

Detection of vitamin D deficiency early in pregnancy has implications for prevention and intervention, yet such studies have only recently been undertaken.[26] Defining the prevalence of vitamin D deficiency in pregnant women and the optimal vitamin D supplementation strategies for these women and their developing infants will help to establish a prototype for recommendations applicable to other communities throughout the United States. A reexamination of dietary vitamin D requirements of various vulnerable populations in the United States, including pregnant women, was recommended by a Cochrane Review in 2000,[83] again in 2012,[84] and by the IOM in their 2010 statement.[9]

Taken together with our findings from the NICHD vitamin D pregnancy study,[7] 4000 IU/d of vitamin D appears to be safe and effective in improving vitamin D levels over time. In addition, increased circulating 25(OH)D levels were associated with reduced occurrence of comorbidities of pregnancy. As such, this study serves as the first step in establishing nor-

FIGURE 5
Longitudinal maternal vitamin D by treatment group: 2000 vs 4000 IU vitamin D/day



Longitudinal total circulating 25-hydroxyvitamin D [25(OH)D] (ng/mL) with group overlay by treatment group, with each participant's 25(OH)D values plotted over time.

Wagner. Vitamin D supplementation during pregnancy. Am J Obstet Gynecol 2012.

mative guidelines for vitamin D supplementation during pregnancy in a diverse group of women in a community health care setting. By evaluating the vitamin D status of pregnant women and their newborn infants who received care at community health centers, our findings serve as a model for other community health care centers in the United States aimed at improving overall health status of its members, including alleviation of the growing problem of hypovitaminosis D throughout the United States. Further, our findings are clinically relevant to community health centers and to those involved in the general health care of pregnant women and their neonates and infants throughout the United States. Additional studies of vitamin D supplementation during pregnancy are warranted to determine optimal dosing and the physiological effects of vitamin D on both the mother and her developing fetus. ■

TABLE 3
Estimated trajectory of serum 25(OH)D according to treatment and race

	2000 IU/d			4000 IU/d		
Month	African American	Hispanic	Caucasian	African American	Hispanic	Caucasian
0	19.2	26.8	30.1	19.0	26.7	30.0
1	24.6	30.1	33.4	25.5	31.0	34.3
2	28.8	32.7	36.1	30.5	34.4	37.9
3	31.6	34.4	38.1	34.2	37.0	40.7
4	33.2	35.4	39.6	36.4	38.6	42.8
5	33.5	35.6	40.4	37.2	39.4	44.1
6	32.5	35.1	40.5	36.6	39.2	44.7

Estimated trajectory of serum 25(OH)D (ng/mL) according to dose group and race, based on quadratic model of serum 25(OH)D over time. Model-based estimates of total circulating 25(OH)D concentrations over time for each race-by-dose subgroup.
25(OH)D, 25-hydroxyvitamin D.

Wagner. Vitamin D supplementation during pregnancy. Am J Obstet Gynecol 2012.

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HB90

Vitamin D for your teeth

Dental Cavities

Dental Cavities occur as a result of **tooth decay**. Tooth decay occurs when **sugars** & **starches** (bread, cereal, milk, soda) are left on your teeth.

Things like brushing, flossing, mouthwash and regular check-ups can help keep teeth clean and cavity free.

Can vitamin D help?

Research shows that vitamin D deficiency can contribute to cavities.

How it works

Vitamin D helps reduce the risk of cavities, by producing **cathelicidin** & **denfensins**. These proteins have **antibacterial** effects to fight bacteria that cause cavities.

Sun exposure & Cavities

Research on **vitamin D, sun exposure, & cavities** found:

74% A 74% reduction in cavities of people who had the most **sun exposure**.

49% And a 49% decrease in cavities among people who took **vitamin D3**.

Children

Studies show children with severe early childhood cavities had much **lower vitamin D levels** than children without severe childhood cavities.

Sources:

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VITAMIN D COUNCIL

Original Article

Comparison of the administration of progesterone versus progesterone and vitamin D in improvement of outcomes in patients with traumatic brain injury: A randomized clinical trial with placebo group

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Abstract

Background: Due to the heterogeneity of traumatic brain injury (TBI), many of single treatments have not been successful in prevention and cure of these kinds of injuries. The neuroprotective effect of progesterone drug on severe brain injuries has been identified, and recently, the neuroprotective effect of vitamin D has also been studied as the combination of these two drugs has shown better effects on animal samples in some studies. This study was conducted to examine the effect of vitamin D and progesterone on brain injury treatment after brain trauma.

Materials and Methods: This study was performed on patients with severe brain trauma (Glasgow Coma Scale (GCS) ≤ 8) from April to September, 2011. The patients were divided to 3 groups (placebo, progesterone, progesterone-vitamin D), each with 20 people. Upon the patients' admission, their GCS and demographic information were recorded. After 3 months, they were reassessed, and their GCS and GOS (Glasgow outcome scale) were recorded. The collected data were analyzed using SPSS 18 software (SPSS Inc., Chicago IL, USA).

Results: Before intervention, GCS mean of the placebo, progesterone, and progesterone-vitamin D groups were 6.3 ± 0.88, 6.31 ± 0.87, and 6 ± 0.88, respectively. They increased to 9.16 ± 1.11, 10.25 ± 1.34, and 11.27 ± 2.27, respectively 3 months after intervention. There was a significant difference among GCS means of the 3 groups (*P-value = 0.001*). GOS was classified to 2 main categories of favorable and unfavorable recovery, of which, favorable recovery in placebo, progesterone, and progesterone-vitamin D was 25%, 45%, and 60%, respectively which showed a statistical significant difference among the groups *(P-value = 0.03)*.

Conclusion: The results showed that recovery rate in patients with severe brain trauma in the group receiving progesterone and vitamin D together was significantly higher than that of progesterone group, which was in turn higher than that of placebo group.

Key Words: Progesterone, severe brain trauma, vitamin D

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INTRODUCTION

Injuries arising from brain trauma are among the causes of death and severe disabilities. Management of brain traumatic injuries includes prevention of neurological complications, intracranial pressure monitoring, and surgical procedures. Finding drugs which are effectively neuroprotective is of special importance for prevention of secondary brain injury after trauma. Attempts have been made, for more than 30 years, to discover an effective and harmless compound which acts as a neuroprotective in brain injuries.[1] During last two decades, all the clinical trials phase II and III for the moderate and severe traumatic brain injuries failed.[1] So far, 130 drugs have been effective on brain injury in animal samples,[2] although they were not effective in clinical trials and do not have any neuroprotective effects.[2,3] One reason is the complexity and diversity of the mechanisms related to different types of TBI, which are not cured by a single drug and cover only one or few receptors.[1,4] Among the studied drugs, progesterone (PROG) has been identified as an effective and safe compound, which is a neuroactive steroidal hormone having neurosteroidal activity in central nervous system.[5-7] In traumatic brain injuries and strokes, this hormone causes blood-brain barrier protection, cerebral edema reduction, inflammatory response, necrosis and apoptosis and stimulation of myelin formation, free radicals reduction, and neuronal loss reduction.[8-11]

Similar to progesterone, vitamin D (VDH) is a neurosteroid acting like a PROG in neuroprotective process.[1] It causes the expression of more than 1000 genes that consequently results in activation of many pathways in CNS (central nervous system).[12] Therefore, VHD in combination with PROG may cure the patients. Recent studies have shown that vitamin D deficiency may intensify traumatic brain injury and reduce the effects of other therapies for TBI. This problem becomes important with respect to the elderly, since, of whom more than 50% suffer from vitamin D deficiency.[13] There are evidences that 30 to 50% of American people suffer from vitamin D deficiency, so that, all the patients with TBI of any age are at risk of unfavorable outcome.[13] Given that the studies examining the effect of the combined PROG and VDH on neural recovery after TBI were most conducted on animals, the present study assessed the effect of these two compounds on outcome improvement in patients with TBI.

MATERIALS AND METHODS

This clinical trial was performed in Alzahra Hospital, Isfahan, Iran, in 2010.

Inclusion criteria of the study

- Patients with brain trauma and diffuse axonal injury
- Patients with GCS < 8

Exclusion criteria of the study

- Patient's legal representative does not consent to patient's participation in the study.

Sampling method

Simple random

Number of samples

20 patients were selected in each group through simple random sampling.

The patients were randomly divided into 3 groups, each with 20 people. Within 8 hours after traumatic injury, the patients in the first group were injected 1 mg/kg of progesterone intramuscularly every 12 hours for 5 days, and the patients in the second group were injected 1 mg/kg of progesterone intramuscularly every 12 hours for 5 days and also 5 μg/kg vitamin D once-a-day for 5 days. The third group as the control group received placebo (Both placebos were injected intravenously). Patients' level of consciousness was periodically controlled based on Glasgow Coma Scale (GCS) during hospitalization and 1 month after treatment, and Glasgow Outcome Scale (GOS) was controlled after 3 months. GOS criteria were defined as follows:

5 = Good recovery: Normal or near normal recovery
4 = Moderate disability: Disable but independent
3 = Severe disability: Dependent with physical / psychological disabilities
2 = Persistent vegetative state
1 = Dead

Finally, these criteria were divided into 2 categories of "favorable" (good recovery and moderate disability) and "unfavorable" (the other 3 criteria).[5] Analysis of the data was done using SPSS 18 software. Qualitative and quantitative data of the groups were compared using Chi square and One-way ANOVA tests, respectively. Comparison of GCS and GOS variations, before and after intervention and among the 3 groups, was done using repeated measurement ANOVA.

RESULTS

This study was carried out in Trauma Center of Alzahra Hospital, Isfahan, Iran, from April to September, 2011. 3 groups of 20 patients were studied and followed up over 3 months.[5] Demographic specifications showed

Table 1: Clinical and demographic characteristics between 3 groups

Admission characteristic	Progesterone	Vitamin D and progesterone	Placebo	*P* value
Males	16 (80%)	16 (80%)	12 (60%)	0.25
Females	4 (20%)	4 (20%)	8 (40%)	0.25
Glasgow Coma scale 3 to 5	5 (25%)	7 (35%)	8 (40%)	0.6
Mechanism of injury				0.9
Motor vehicle	8 (40%)	7 (35%)	8 (40%)	
Fall	3 (15%)	3 (15%)	2 (10%)	
Assault	1 (5%)	4 (20%)	3 (15%)	
Car accident	6 (30%)	4 (20%)	5 (25%)	
Others	2 (10%)	2 (10%)	2 (10%)	
Surgical procedure	6 (30%)	8 (40%)	9 (45%)	0.7
Mean (standard deviation) age	28 (7.43)	28.3 (8.84)	31.45 (8.17)	0.3
Mean qualifying Glasgow coma scale score	6 (1.07)	5.8 (1.15)	5.8 (1.19)	0.8
Mean time injury to admission (hours)	3.9 (1.88)	4.7 (6.39)	3.5 (1.73)	0.6

Table 2: Comparison of Glasgow outcome Scale scores between the progesterone, progesterone –Vitamin D, and placebo groups at 3 months later

Glasgow Outcome Scale Score	Progesterone (%)	Vitamin D and progesterone (%)	Placebo (%)
Good recovery	5 (25)	7 (35)	3 (15)
Moderate disability	4 (20)	5 (25)	2 (10)
Severe disability	4 (20)	3 (15)	3 (15)
Vegetative	3 (15)	3 (15)	4 (20)
Death	4 (20)	2 (10)	8 (40)

no significant difference among the 3 groups, i.e., the patients were matched in this regard [Table 1].

Before intervention, GCS mean of the placebo, progesterone, and progesterone-vitamin D groups comprised 6.3 ± 0.88, 6.31 ± 0.87, and 6 ± 0.88, respectively, which 3 months after intervention, increased to 9.16 ± 1.11, 10.25 ± 1.34, and 11.27 ± 2.27, respectively. There was a significant difference among GCS means of the 3 groups (*P*-value = 0.001) [Figure 1].

GOS values of the patients, 3 months after trauma, are shown in Table 2. Recovery rate in the group receiving progesterone and vitamin D together was higher than that of other groups; consequently, mortality rate in this group was less than that of others (*P*-value = 0.03).

Favorable responses in placebo, progesterone, and progesterone-vitamin D comprised 25%, 45%, and 60%, respectively which showed a statistical significant difference among the groups (*P*-value = 0.03) [Figure 2].

In this study, mean mortality rate was 23.3% (14 patients) as the mortality rate in the placebo, progesterone-vitamin D, and progesterone groups was 40% (8 patients), 10% (2 patients), and 20% (4 patients), respectively. These rates showed a significant difference among groups (*P*-value = 0.000).

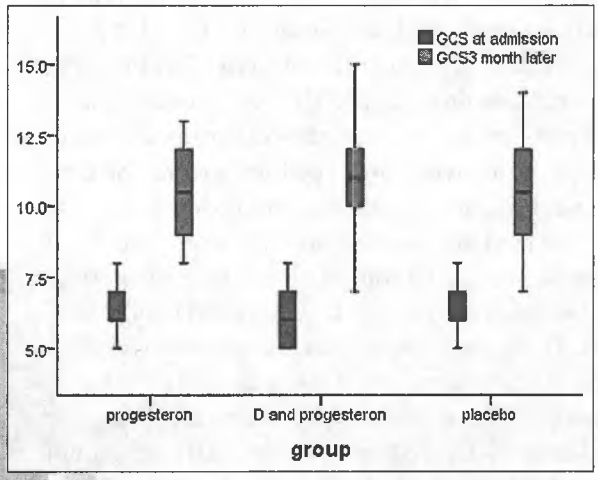

Figure 1: Comparison of GCS at administration and 3 month later between 3 groups

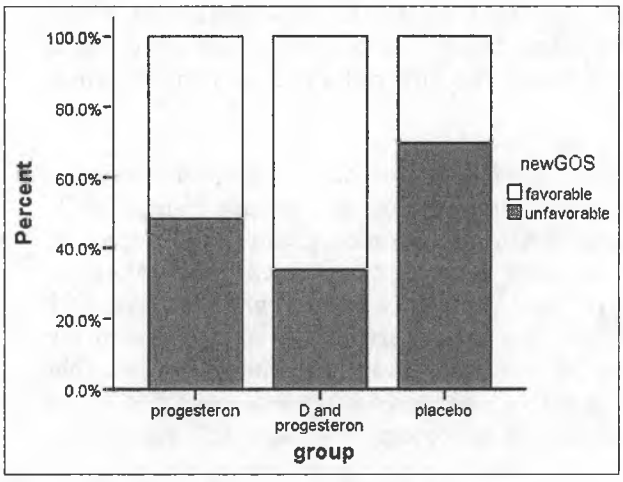

Figure 2: Comparison of dichotomized Glasgow Outcome scale score for patients receiving placebo or progesterone or progesterone and vitamin D after 3 month

DISCUSSION

Numerous studies have been done on TBI treatment.

However, most of them were conducted under *in vitro* and *in vivo* conditions. There have been also few studies on TBI treatment with combined progesterone and vitamin D, especially on human samples. In this study, progesterone and progesterone-vitamin D were found to improve the patients' outcome as the recovery rate in the group with combination therapy was significantly more than that in other groups. Moreover, GCS variations and mortality rate in the group with combination therapy were significantly less than those in other groups. Favorable outcome of the group with combination therapy was significantly more than those in other groups. A similar study performed under *in vitro* condition[1] showed that low dose PROG (0.01, 0.1, and 1.5 μmol/l) did not reduce glutamate-induced cell death, whereas with higher doses (10, 24, 40, and 80 μmol/l), PROG significantly reduced LDH and MTT as the optimum dose of PROG was reported as 20 μmol/l. Furthermore, therapeutic responses to vitamin D were found to have a U-shaped pattern as the most favorable response was found in low doses (0.001 - 0.5 μmol/l), and the least neuroprotective effect was found in higher doses (1 - 10 μmol/l).[1] In the above study, the combination of vitamin D (0.1 μmol/l) and PROG (20 μmol/l) showed no significant preventive effect than the other group, and this was contrary to the U-shaped pattern of response to vitamin D; however, the combined PROG (20 μmol/l) and VDH (20 μmol/l) significantly reduced cell death and was more effective than any of the drugs alone.

Another study on the effect of progesterone alone showed more favorable outcome and much less mortality rate for the patients in progesterone group.[5]

The effect of progesterone on σ1 receptor acts as a competitive inhibitor and may reduce N-methyl-D-aspartate (NMDA) glutamate signaling.[14,15] Moreover, PROG affects nicotinic acetylcholine receptor (nAChR)[16] and stimulates gamma-aminobutyric acid (GABA) as the most important inhibition transmitter in brain. All the above 3 mechanisms are responsible for the positive neuroprotective effects of PROG as they inhibit the excitotoxic responses in injuries.[13]

Vitamin D belongs to the secosteroid class, which directly causes the expression of more than 100 genes.[17] This neuroactive steroid can be dispersed in CNS since it is the final activator of enzymes and has an intracellular receptor. The primary effects of vitamin D are the inhibition of cell proliferation and stimulation of cellular differentiation, especially in immune system.[18,19] It has been found that vitamin D deviates all the dimensions of immune performance, which is near the immune response type 2 and generally is anti-inflammatory and regulatory.[13] Similar to progesterone, vitamin D reduces the pro-inflammatory level of TH1 cytokines such as IL6, IL12, IL1B, and TNFα.[20] Furthermore, vitamin D maintains the intracellular surface of calcium through following ways: 1. Maintenance of PTH at an appropriate level, 2. Adjustment of L-type voltage-sensitive calcium channels, and 3. Control of intracellular Ca^{2+} buffering.

The progesterone activity through GABAergic system to inhibit extracellular activity and the performance of vitamin D to increase intracellular Ca^{2+}-binding proteins together affect the calcium metabolism through sub-pathways.

Other reasons for the use of combined PROG and vitamin D in TBI treatment are as follows:

- Reduction of the effects of glutamate release and calcium inflex
- Protection against toxic effects of heme breakdown products
- Enhancement of free radical scavenging
- Modulation of the renin-angiotensin system
- Protection of the axonal and cytoskeleton infrastructure

CONCLUSION

As mentioned before, vitamin D and progesterone are pleiotropic hormones with a lot of common pathways, which consequently reduce the CNS injury and increase recovery rate of nervous system after TBI. Many studies performed on mice and humans have reported significant favorable outcome after TBI.[21-23] Recently, progesterone has been found to reduce inflammatory response and oxidative stress.[24,25] Moreover, progesterone activates the protective pathways and increases the expression of genes and proteins related to neuroprotection after TBI.[13] There are also reports about the neuroprotective effect of vitamin D under *in vitro* and *in vivo* conditions and cortical infarcts.[26] Furthermore, studies have shown that vitamin D deficiency disrupts the processes associated with CNS health such as mitosis, mitogenesis, neurite outgrowth, possibly adult neurogenesis in hippocampal cells, and mitochondria function.[27]

Regarding the foregoing, the use of combined PROG and vitamin D is reasonable in that vitamin D in combination with PROG improves repair mechanisms of CNS considering their common pathways, and also compensates other mechanisms, which are not

performed by PROG. This reduces the heterogeneity of TBI and the probable failure of a single treatment.

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ALASKA STATE LEGISLATURE PASSES VITAMIN D AWARENESS RESOLUTION

VITAMIN D-HCR 5

MOVING TOWARD A PREVENTION OF DISEASE MODEL FOR HEALTH CARE

Scientific studies demonstrate the incidence of disease for over 20 conditions can be significantly reduced with vitamin D serum levels above 40 ng/ml.

Decrease your risk of

- > Cancer
- > Diabetes
- > Heat Attack
- > Arthritis
- > SAD
- > Periodontal Disease
- > Multiple Sclerosis
- > Rickets
- > Fractures
- > Falling
- > Premature Birth
- > Preeclampsia

Contact Representative Seaton
907-235-2921
Rep.Paul.Seaton@legis.state.ak.us

The House and the Senate of the Alaska State Legislature unanimously passed HCR 5 this session, a resolution supporting prevention of disease as a priority for the State and encouraging supplementation of vitamin D for its long term preventative health benefits. Hundreds of peer reviewed scientific studies show the health benefits of having vitamin D serum levels above 40 nanograms per milliliter. Although we make vitamin D naturally, Alaskans don't get enough vitamin D due to our northern latitude and modern diet. Supplements are a cheap way to get adequate vitamin D. Everyone processes vitamin D differently, so it is important to have a blood test to determine your levels. Doctors recommend levels between 40-80 ng/ml.

Read more about vitamin D at grassrootshealth.net or vitamindcouncil.org

CS FOR HOUSE CONCURRENT RESOLUTION NO. 5(HSS)
IN THE LEGISLATURE OF THE STATE OF ALASKA
TWENTY-SEVENTH LEGISLATURE - FIRST SESSION
BY THE HOUSE HEALTH AND SOCIAL SERVICES COMMITTEE

Offered: 3/16/11

Go to housemajority.org/seaton select "Vitamin D Studies" and click on a **WHEREAS** to view the journal article.

A RESOLUTION

Relating to prevention of disease and to vitamin D.
BE IT RESOLVED BY THE LEGISLATURE OF THE STATE OF ALASKA:

WHEREAS the nutrient and pre-hormone vitamin D is manufactured in the skin during exposure to ultraviolet B light from high-angle sunshine; and

WHEREAS, for seven months a year, the angle of the sun's rays is too low for adequate ultraviolet B exposure in the state; and

WHEREAS Alaskans have one of the lowest levels of vitamin D blood serum in the nation because of the state's northern latitude; and

WHEREAS the state has a high incidence of preventable diseases that numerous studies indicate may be correlated with insufficient blood serum levels of vitamin D; and

WHEREAS a 2008 study by the Ketchikan Indian Community Tribal Health Clinic found that blood serum levels of vitamin D of Alaska Natives tested in Ketchikan averaged between 6 and 17 ng/ml; and

WHEREAS a 1986 study by the University of Alaska Fairbanks found the bloods Serum levels of vitamin D of Caucasian males averaged 27 ng/ml; and

WHEREAS a 2007 article published in the American Journal of Clinical Nutrition reported that a study that compared cancer rates of a group of postmenopausal women taking 1,100 IU of vitamin D supplements in combination with calcium to cancer rates of a group taking a placebo found the risk of developing any cancer after four years was 60 percent lower in the group taking vitamin D supplements; and

WHEREAS a study presented at the 2008 annual meeting of the American Association for Cancer Research found that blood serum levels of vitamin D of at least 50 ng/ml were associated with an 83 percent reduction in the incidence of breast cancer compared to blood serum levels of vitamin D of 25 ng/ml; and

WHEREAS a 2007 article published in the American Journal of Preventative

Medicine reported that a study found that a group with blood serum levels of vitamin D of at least 42 ng/ml had a 60 percent reduction in the incidence of colorectal cancer compared to a group with blood serum levels of vitamin D of 25 ng/ml; and

WHEREAS a 2010 study by the University of San Diego showed that incidence of bladder cancer increases as latitude increases and that the incidence of bladder cancer decreased by 40 percent with adequate blood serum levels of vitamin D; and

WHEREAS a study referenced by Michael F. Holick, Ph.D., M.D., in The Vitamin D Solution found that men with prostate cancer who received 2,000 IU of vitamin D a day for two years had a 50 percent reduction in the rise of prostate-specific antigen, an indicator of prostate cancer activity; and

WHEREAS a 2001 study published in The Lancet found that a group with blood serum levels of vitamin D of 52 ng/ml had a 66 percent reduction in the incidence of type 1 diabetes compared to a group with blood serum levels of vitamin D of 25 ng/ml; and

WHEREAS a 2001 study published in the Lancet found that children in Finland who received 2,000 IU a day of vitamin D for the first year of life were 80 percent less likely to develop type 1 diabetes by age 30 compared to children receiving 400 IU a day of vitamin D; and

WHEREAS a 2006 study published in Diabetes Care found that taking 800 IU of vitamin D in combination with calcium resulted in a 33 percent reduction in the risk of type 2 diabetes; and

WHEREAS a 1998 study published in the Journal of the American College of Cardiology found that the incidence of heart attacks is 53 percent higher during the sun 27- deprived winter months than during the summer months; and

WHEREAS a growing body of research from around the world indicates that deficiency in vitamin D correlates with a broad spectrum of conditions, such as high blood pressure, poor insulin sensitivity, inflammation, and other conditions related to heart disease; and

WHEREAS numerous studies have found that vitamin D suppresses the inflammation that plays a role in rheumatoid arthritis, chronic muscle pain, metabolic syndrome, congestive heart failure, and stroke; and

WHEREAS a 2008 study published in the Archives of Internal Medicine showed that the risk for heart attack in men with vitamin D blood serum levels at or below 15 ng/ml is 2.4 times greater than that for men whose vitamin D levels are at or above 30 ng/ml; and

WHEREAS a 1999 study published in the Journal of Nutrition, Health and Aging 13 found that patients with seasonal affective disorder treated with a single dose of 100,000 IU

of vitamin D showed significant improvement after one month; and

WHEREAS a 2004 study published in the American Journal of Clinical Nutrition found that low blood serum levels of vitamin D were associated with periodontal disease; and

WHEREAS a 2005 study published in the American Journal of Public Health found that the rate of oral disease among Alaska Natives is disproportionately high; and

WHEREAS a 2010 study published in the Journal of Laryngology and Otology found that low levels of vitamin D are associated with an increased incidence of upper respiratory tract infections; and

WHEREAS the Centers for Disease Control and Prevention report that influenza vaccine effectiveness varies greatly; and

WHEREAS in 2010, the Department of Health and Social Services, reported that the state is no longer subsidizing universal vaccinations for influenza because of a seven-fold increase in cost over 10 years and a decrease in federal funding; and

WHEREAS a 2010 article published in the American Journal of Clinical Nutrition 28 reported that a study of a group of Japanese school children who received 1,200 IU of vitamin D a day showed a 50 percent reduction in the incidence of influenza compared to other school children; and

WHEREAS vitamin D has been shown to influence the immune response to tuberculosis, and studies have shown that vitamin D deficiency is associated with increased risk of acquiring tuberculosis; and

WHEREAS a 2010 article in The Lancet reported that the risk of multiple sclerosis increases with latitude and with low blood serum levels of vitamin D; and

WHEREAS a 2006 article published in the Journal of American Medical Association reported that a study examining blood samples of more than 7,000,000 army recruits from 1992 - 2004 found that higher blood serum levels of vitamin D were associated with a significantly lower risk of developing multiple sclerosis; and

WHEREAS a 2005 article published in the Journal of the American Medical Association reported that elderly persons who had blood serum levels of vitamin D of at least 45 ng/ml experienced a 50 percent reduction of fractures, and a 2007 article published in the Journal of the American Geriatric Society reported that elderly persons who had blood serum levels of vitamin D of at least 30 ng/ml experienced a 72 percent reduction in falls compared to those who had blood serum levels of vitamin D below 25 ng/ml; and

WHEREAS the elderly are at high risk for vitamin D deficiency because of indoor lifestyle and the reduced ability of aging skin to manufacture vitamin D; and

WHEREAS a 2009 article published in the Journal of Alzheimer's Disease reported that vitamin D reduces the risk of several types of diseases that have been identified as risk factors for or precursors to dementia; and

WHEREAS a 2010 article published in The Journal of Alternative and Complementary Medicine reported that a study in Egypt found that children without autism had blood serum levels of vitamin D averaging 40.1 ng/ml, and children with autism had significantly lower blood serum levels of vitamin D, averaging 28.5 ng/ml; and

WHEREAS Sara B. Arnaud, M.D., found that infants and children with blood serum levels of vitamin D of at least 18 ng/ml have a 99 percent prevention rate of the bone disease rickets; and

WHEREAS a 2007 study published in the Journal of Clinical Endocrinology and Metabolism found that females who received regular vitamin D supplementation during the first year of life are 50 percent less likely to develop preeclampsia in their first pregnancy; and

WHEREAS a 2009 article published in The Journal of Clinical Endocrinology and Metabolism found that pregnant women with low blood serum levels of vitamin D were nearly four times more likely to deliver by cesarean section than women with blood serum levels of vitamin D of at least 15 ng/ml; and

WHEREAS a 2009 study at the Medical University of South Carolina found that pregnant women who took 4,000 IU a day of vitamin D during pregnancy had a 50 percent reduction in the rate of premature birth and delivered fewer babies with low birth weight than women who took 400 IU a day of vitamin D; and

WHEREAS a 2010 study at the Rebecca Sieff Hospital in Israel found that when patients with hepatitis C were given 1,000 IU a day of vitamin D, the blood of 44 percent of the participants was virus-free after a month of treatment, and the blood of 96 percent of the participants was virus-free after three months; and

WHEREAS, although the Institute of Medicine of the National Academy of Sciences, in 2010, recommended 600 IU a day of vitamin D, levels above 2,000 IU a day and an upper level intake of 4,000 IU a day may be more appropriate for those who live in the northern latitude; and

WHEREAS a 2007 study published in the American Journal of Clinical Nutrition found vitamin D toxicity only above 30,000 IU a day; and

WHEREAS a 2007 article published in the Journal of Photochemistry and Photobiology estimated that the United States economic burden due to vitamin D deficiency from inadequate exposure to ultraviolet B light, inadequate diet, and lack of supplements was estimated at $40,000,000,000 - 56,000,000,000 in 2004; and

WHEREAS a 2010 article published in Molecular Nutrition and Food Research regarding the rate of premature death and the economic burden in Canada found that annual deaths could be reduced by 37,000 and the economic burden reduced by 6.9 percent or $14,400,000,000 if blood serum levels of vitamin D of the population were adequate; and

WHEREAS part of the budget of the Department of Health and Social Services is used to treat illnesses that could potentially be prevented with adequate blood serum levels of vitamin D; and

WHEREAS the above-referenced studies and findings taken in aggregate provide significant evidence for the benefits of vitamin D supplements; and

WHEREAS vitamin D supplements are inexpensive;

BE IT RESOLVED that the Alaska State Legislature respectfully requests the Governor to establish prevention of disease as a primary model of health care in Alaska; and be it

FURTHER RESOLVED that the Alaska State Legislature encourages the Alaska Department of Health and Social Services and health care providers to increase attention to vitamin D deficiency and vitamin D blood testing and to promote awareness of the potential long-term health benefits of and increased chances of cancer survival with sufficient levels of vitamin D; and be it

FURTHER RESOLVED that the Alaska State Legislature urges the Department of Health and Social Services to

(1) promote vitamin D supplements for the elderly potentially to prevent bone loss, falls, fractures, and other age-related health problems;

(2) determine the relative effectiveness of influenza vaccination as compared with vitamin D supplementation, using the comparative treatment effectiveness analysis;

(3) investigate substituting vitamin D supplementation as a cost-effective method for preventing influenza in the adult population not identified as high risk; and

(4) promote vitamin D supplements for pregnant women and infants to prevent pregnancy complications, preterm births, type 1 diabetes, and rickets.

Vitamin D

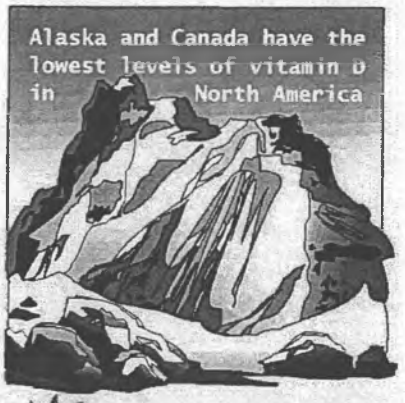
Dosage Recommendations from medical experts

"The body needs at least 4000 IU/day in order to maintain a healthy concentration of vitamin D in the blood." - Robert P. Heany, MD Creighton Uni-

"My advice, especially for pregnant women: continue taking 5,000 IU/day until your 25(OH)D is between 50-80" *Source: John Jacob Cannell MD, Vitamin D Council ED*

For more information on recommendations and guidelines visit
Grassrootshealth.net
VitaminDcouncil.org
Housemajority.org/seaton

Each body processes vitamin D differently and has different levels of sun exposure. Consult a health care practitioner to develop a custom plan that meets your specific vitamin D needs.



Science shows that vitamin D sufficient populations have reduced relative risk.

- 50% less Breast Cancer
- 50% less Colorectal Cancer
- 40% less Bladder Cancer
- 33% less Type 2 Diabetes
- 2.4 times fewer Heart Attacks
- 50% less Influenza & Upper Respiratory Disease
- 70% fewer Falls in Elderly
- 50% fewer Bone Fractures

All for less than $7 per person per year!
(500 soft gels at 5000 IU ea for $8.99)

Distributed by State Representative Paul Seaton * 907-235-2921