DOI: 10.1111/j.1471-0528.2012.03495.x
www.bjog.org

Vitamin D status and recurrent preterm birth: a nested case–control study in high-risk women

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Accepted 5 August 2012. Published Online 19 October 2012.

Objective To determine whether vitamin D status is associated with recurrent preterm birth, and any interactions between vitamin D levels and fish consumption.

Design A nested case–control study, using data from a randomised trial of omega-3 fatty acid supplementation to prevent recurrent preterm birth.

Setting Fourteen academic health centres in the USA.

Population Women with prior spontaneous preterm birth.

Methods In 131 cases (preterm delivery at <35 weeks of gestation) and 134 term controls, we measured serum 25-hydroxyvitamin D [25(OH)D] concentrations by liquid chromatography-tandem mass spectrometry (LC-MS) from samples collected at baseline (16–22 weeks of gestation). Logistic regression models controlled for study centre, maternal age, race/ethnicity, number of prior preterm deliveries, smoking status, body mass index, and treatment.

Main outcome measures Recurrent preterm birth at <37 and <32 weeks of gestation.

Results The median mid-gestation serum 25(OH)D concentration was 67 nmol/l, and 27% had concentrations of <50 nmol/l. Serum 25(OH)D concentration was not significantly associated with preterm birth (OR 1.33; 95% CI 0.48–3.70 for lowest versus highest quartiles). Likewise, comparing women with 25(OH)D concentrations of 50 nmol/l, or higher, with those with <50 nmol/l generated an odds ratio of 0.80 (95% CI 0.38–1.69). Contrary to our expectation, a negative correlation was observed between fish consumption and serum 25(OH)D concentration (−0.18, $P < 0.01$).

Conclusions In a cohort of women with a prior preterm birth, vitamin D status at mid-pregnancy was not associated with recurrent preterm birth.

Keywords Perinatal nutrition, preterm birth, vitamin D.

Please cite this paper as: Thorp J, Camargo C, McGee P, Harper M, Klebanoff M, Sorokin Y, Varner M, Wapner R, Caritis S, Iams J, Carpenter M, Peaceman A, Mercer B, Sciscione A, Rouse D, Ramin S, Anderson G. Vitamin D status and recurrent preterm birth: a nested case–control study in high-risk women. BJOG 2012;119:1617–1623.

Introduction

Vitamin D has multiple functions that are critical in growth and development.[1] The best marker of vitamin D status is the circulating concentration of its metabolite 25-hydroxyvitamin D [25(OH)D]. When serum 25(OH)D concentrations have been measured in cohorts of pregnant women in the USA, many women from various ethnic

groups living at different latitudes are found to have a low vitamin D status, regardless of the exact definition used.[2] Low maternal concentrations of 25(OH)D have been associated with severe pre-eclampsia and low birthweight in some studies, but not in others.[3–9]

In a randomised trial of omega-3 fatty acid supplementation in pregnant women with a history of preterm birth, our group observed an overall recurrent preterm birth rate of 40%, and found that although omega-3 fatty acid supplementation did not reduce the risk of recurrent preterm birth (relative risk, RR 0.91; 95% CI 0.77–1.07),[10] the self-reported consumption of fish was protective.[11] This probably represents unmeasured confounding, as fish is the major dietary source of omega-3 fatty acids. Because fish is a major dietary source of vitamin D, we conducted a secondary analysis in this cohort to examine whether vitamin D status was associated with recurrent preterm birth. We then explored whether vitamin D status was correlated with the consumption of fish and mediated the protective association between fish consumption and recurrent preterm birth.

Methods

This is an observational study, with a secondary analysis of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Maternal–Fetal Medicine Units (MFMU) Network randomised clinical trial of omega-3 long chain polyunsaturated fatty acid (LCPUFA) supplementation to prevent recurrent preterm birth. Trial investigators recruited women with a history of at least one previous spontaneous singleton preterm birth at 13 network centres from January 2005 to October 2006.[10] A total of 434 women were randomised to receive daily supplementation of 1200 mg eicosapentaenoic acid (EPA, 20:5n-3) and 800 mg of docosahexaenoic acid (DHA, 22:6n-3), and 418 women were assigned to matching placebos, beginning at 16–21 6/7 weeks of gestation, and continuing until 36 6/7 weeks of gestation or delivery, whichever occurred first. As part of the trial, all enrolled women also received weekly injections of 17α-hydroxyprogesterone caproate. Women currently taking fish oil or omega-3 PUFA supplements were ineligible for the trial; detailed inclusion and exclusion criteria are reported elsewhere.[10] The study (NCT00135902 at www.clinicaltrials.gov) was approved by the institutional review boards (IRBs) of the biostatistical coordinating centre and all participating clinical centres, and this secondary analysis was determined to be exempt from IRB review of the Office of Human Subjects by the IRB office at the University of North Carolina, Chapel Hill, NC, USA. All enrolled women gave written informed consent. The CONSORT flow sheet and checklist for the omega-3 trial can be found in the article in which the primary trial results were published.[10]

The current analysis is a nested case–control study in which patients that delivered at or beyond 37 weeks of gestation were selected as controls and matched on race/ethnicity and study site in an approximate 1:1 ratio with cases, defined as delivery before 35 weeks of gestation. The cut-off point of 35 weeks of gestation was chosen as an inclusion criterion to enrich the total number of preterm births at <37 and 32 weeks of gestation in the clinical trial. The reader should note as they read our results that the gestational age limit defining the inclusion criteria differed from the outcome. This analysis is restricted to patients that consented to the use of their blood for future research on prematurity and other pregnancy complications. Outcomes assessed included all preterm births (<37 weeks of gestation) and very early preterm births (<32 weeks of gestation) in the subsequent pregnancy.

To characterise the women's vitamin D status upon enrollment to the trial, we measured 25(OH)D concentrations using liquid chromatography-tandem mass spectrometry (LC-MS) from serum collected at the baseline randomisation visit (at 16–22 weeks of gestation) in 131 cases and 134 controls. Serum 25(OH)D was also measured in follow-up samples collected at 25–28 weeks of gestation in a subset of 80 cases and 88 controls. The method used is an isotope dilution, LC-MS assay that has been optimised in the Massachusetts General Hospital laboratory, based on published procedures.[12] The limit of detection is 5 nmol/l for D2 and 7.5 nmol/l for D3. The between-run comparison value for a quality control serum containing a total vitamin D concentration of 57 nmol/l is 7.5%. 25(OH)D measurements are robust even when frozen, and are not altered by exposure to light. For the purpose of this analysis 25(OH)D concentrations were examined as quartiles (based on the distribution of controls) to assess dose response, and the results were dichotomised at the level of 50 nmol/l, as concentrations below this level are considered 'inadequate' by the Institute of Medicine.[13]

The exact date of the solstice or equinox in each given year was used to define the season (winter, spring, summer, fall) when the serum was collected for 25(OH)D. Fish consumption was categorised as none, once or twice per week, or three or more times per week, and was based on the self-reported baseline intake during the current pregnancy of dark-meat fish, canned tuna, other fish and shellfish.

To examine the association between baseline 25(OH)D concentration and recurrent preterm birth (yes/no), we used conditional logistic regression models to control for race/ethnicity, study centre, maternal age, number of prior preterm deliveries, smoking status, body mass index (BMI), season when the measurement was made and treatment group, which were chosen *a priori* based on clinical relevance. A locally weighted scatter plot smoothing technique (loess) was used to assess the full range of 25(OH)D

concentration against the logit of preterm birth. The point biserial correlation was used to assess the relationship between serum 25(OH)D concentration and the number of fish servings per week. Two-sided $P < 0.05$ was considered to be statistically significant.

Results

Baseline characteristics are presented in Table 1. The median 25(OH)D concentration was 67 nmol/l at 16–22 weeks of gestation and 76 nmol/l at 25–28 weeks of gestation. Only 22% of participants had 25(OH)D concentrations <50 nmol/l at mid-gestation. One hundred and sixty subjects had vitamin D levels measured at both time points. Using a paired Student's *t*-test to compare means at different times, vitamin D levels were higher at the second visit (mean difference 5.7 nmol/l; $P < 0.0001$). We also compared medians at the different time points for the whole sample (acknowledging that some women delivered prior to the second visit, and thus could bias results), but the difference in medians was not significant ($P = 0.053$). Table 2 shows the baseline characteristics by mid-gestation serum 25(OH)D concentration, dichotomised at 50 nmol/l. The correlation between serum 25(OH)D concentration and fish consumption was −0.18 ($P < 0.01$); therefore, a formal mediation analysis was not conducted.

Table 1. Baseline characteristics

	Cases (<35 weeks of gestation), *n* = 131	Controls (≥37 weeks of gestation), *n* = 134	*P*
Age (years)	26.8 ± 5.5	27.3 ± 5.6	0.54
Race/ethnicity			
Black	53 (40%)	50 (37%)	0.73
Hispanic	16 (12%)	14 (10%)	
White	62 (47%)	70 (52%)	
Number of prior preterm deliveries			
1	79 (60%)	108 (81%)	<0.0001
2	39 (30%)	24 (18%)	
3 or more	13 (10%)	2 (1%)	
Smoking during pregnancy	32 (24%)	13 (10%)	0.002
Pre-pregnancy BMI (kg/m^2)	26.8 ± 7.2	26.4 ± 6.1	0.91
Study centre region			
Northern USA*	96 (73%)	99 (74%)	1.0
Southern USA**	35 (27%)	35 (26%)	
Season of blood draw for 25(OH)D			
Winter	15 (11%)	26 (19%)	0.34
Spring	50 (38%)	45 (34%)	
Summer	41 (31%)	41 (31%)	
Fall	25 (19%)	22 (16%)	
Number of times fish eaten/week			
None	53 (40%)	33 (25%)	0.008
1 or 2	58 (44%)	84 (63%)	
3+	20 (15%)	17 (13%)	
25(OH)D concentration (nmol/L)	70.7 ± 30.7	72.7 ± 32.6	0.61
Assigned to omega-3 group	63 (48%)	72 (54%)	0.40

Continuous variables are presented as means ± standard deviations, and the *P* values are reported from the Wilcoxon test. Categorical variables are presented as frequencies, and the *P* values are reported from the Fisher's exact test.
*Northern sites: Utah, Illinois, Michigan, Ohio, Pennsylvania, New York, and Rhode Island.
**Southern sites: North Carolina, Alabama, and Texas.

Table 2. Baseline characteristics of women, ranked by mid-gestation serum 25(OH)D concentration

	25(OH)D < 50 nmol/l, *n* = 71	25(OH)D ≥ 50 nmol/l, *n* = 194	*P*
Age (years)	24.7 ± 5.0	27.9 ± 5.5	<0.0001
Race/ethnicity			
Black	52 (73%)	51 (26%)	<0.0001
Hispanic	14 (20%)	16 (8%)	
White	5 (7%)	127 (65%)	
Number of prior preterm deliveries			
1	54 (76%)	133 (69%)	0.26
2	12 (17%)	51 (26%)	
3 or more	5 (7%)	10 (5%)	
Smoking during pregnancy	17 (24%)	28 (14%)	0.095
Pre-pregnancy BMI (kg/m^2)	29.6 ± 8.3	25.5 ± 5.6	0.0002
Study centre region			
Northern USA*	38 (54%)	157 (81%)	<0.0001
Southern USA**	33 (46%)	37 (19%)	
Season of blood draw for 25(OH)D			
Winter	17 (24%)	24 (12%)	0.0316
Spring	28 (39%)	67 (35%)	
Summer	14 (20%)	68 (35%)	
Fall	12 (17%)	35 (18%)	
Number of times fish eaten/week			
None	13 (18%)	73 (38%)	0.0090
1 or 2	45 (63%)	97 (50%)	
3+	13 (18%)	24 (12%)	
25(OH)D concentration (nmol/l)	34.1 ± 10.3	85.5 ± 24.9	<0.0001
Assigned to omega-3 group	34 (48%)	101 (52%)	0.58

*Northern sites: Utah, Illinois, Michigan, Ohio, Pennsylvania, New York, and Rhode Island.
**Southern sites: North Carolina, Alabama, and Texas.

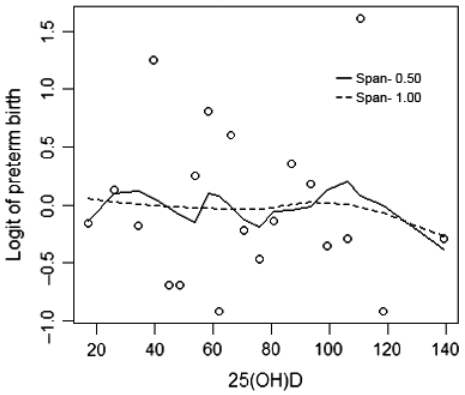

Figure 1. Relationship between mid-gestation serum 25(OH)D concentration and repeat preterm birth, using the LOESS smoother. Circles represent each median value of 20 groups, ranked by 25(OH)D.

Low vitamin D status at 16–22 weeks of gestation was not associated with recurrent preterm birth (Figure 1; Table 3). In the univariate relationship with preterm birth (Table 1), the number of prior preterm deliveries, smoking status, and fish intake were found to be significantly associated. These remained significant in the fully adjusted model. Similar null findings were observed for vitamin D status at 25–28 weeks of gestation (data not shown). Analyses were repeated for the 70 preterm births at <32 weeks of gestation, and again there was no association between low vitamin D status and very early preterm birth.

Our study participants had a marked racial disparity in vitamin D deficiency (<50 nmol/l) at 16–22 weeks of gestation. Of the self-reported African American women, 52% were deficient in vitamin D, versus only 27% of the total cohort. We did a subgroup analysis looking at just recurrent preterm birth in African American women and found no association with vitamin D deficiency (Table 4).

Discussion

Given the mixture of positive and negative associations previously reported between vitamin D status and poor pregnancy outcomes (e.g. severe pre-eclampsia),[1,3–9] we wanted to explore the hypothesis that low vitamin D status would be associated with recurrent preterm birth. We found no evidence of such a relationship. Although our null findings may reflect the underlying biology in this high-risk population, one should keep in mind that the majority of our mothers did not have serum 25(OH)D concentrations of <50 nmol/l, and prior spontaneous preterm birth was an entry criterion for the omega-3 supplementation trial. This entry criterion selected against women with histories of pre-eclampsia (another pathway to early delivery), the perinatal condition that is most often linked to low vitamin D status.[5]

Our results differ from prior observational studies of vitamin D status in pregnancy, which have reported that low vitamin D status was common;[1] we found that participants in this clinical trial to prevent recurrent preterm birth had, for the most part, concentrations of 50 nmol/l or higher. Because the human fetus is entirely dependent on the maternal pool of vitamin D, these previous reports of hypovitaminosis D have caused public health officials and nutritionists to question whether the recommended vitamin D intake in pregnancy should be increased.[1] Our results from a diverse population of women cared for in

Table 3. Association between mid-gestation serum 25(OH)D concentrations and recurrent preterm birth

	Quartiles of 25(OH)D measured at 16–22 weeks of gestation				25(OH)D concentration at 16–22 weeks of gestation	
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	<50 nmol/l	≥50 nmol/l
n	63	72	62	68	71	194
Median 25(OH)D concentration (nmol/l)	35	57	82	110	35	82
Range of 25(OH)D concentrations (nmol/l)	(10, 45)	(47, 67)	(70, 95)	(97, 167)	(10, 47)	(50, 167)
Preterm birth, *n* (%)	33 (52%)	37 (51%)	31 (50%)	30 (44%)	35 (49%)	96 (49%)
Model 1* OR (95% CI)	1.43 (0.60–3.41)	1.38 (0.66–2.91)	1.27 (0.62–2.60)	1.00 (referent)	0.87 (0.46–1.66)	1.00 (referent)
Model 2** OR (95% CI)	1.28 (0.47–3.49)	1.23 (0.53–2.87)	1.30 (0.60–2.84)	1.00 (referent)	0.82 (0.40–1.70)	1.00 (referent)
Model 3*** OR (95% CI)	1.33 (0.48–3.70)	1.25 (0.53–2.97)	1.31 (0.60–2.87)	1.00 (referent)	0.80 (0.38–1.69)	1.00 (referent)

*From conditional logistic regression, controlling for the matching variables only (race/ethnicity and study center).
***From conditional logistic regression, controlling for the matching variables (race/ethnicity and study center) and maternal age, number of prior preterm deliveries, smoking status, BMI, season when blood was drawn, and treatment group.
***From conditional logistic regression, controlling for all variables in model 2 plus fish intake.

Table 4. Association between mid-gestation serum 25(OH)D concentrations and recurrent preterm birth in the African American subgroup only

	Quartiles of 25(OH)D concentration measured at 16–22 weeks of gestation				25(OH)D concentration at 16–22 weeks of gestation	
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	<50 nmol/l	≥50 nmol/l
n	21	31	15	36	52	51
Median 25(OH)D concentration (nmol/l)	22	40	55	75	35	67
Range of 25(OH)D concentrations (nmol/l)	(10, 30)	(32, 47)	(50, 65)	(62, 112)	(10, 47)	(50, 112)
Preterm birth, *n* (%)	9 (43%)	18 (58%)	7 (47%)	19 (53%)	27 (52%)	26 (51%)
Model 1* OR (95% CI)	0.64 (0.21–2.02)	1.17 (0.44–3.12)	0.70 (0.20–2.45)	1.00 (referent)	1.06 (0.49–2.30)	1.00 (referent)
Model 2** OR (95% CI)	0.54 (0.14–2.00)	1.01 (0.32–3.14)	0.46 (0.11–0.97)	1.00 (referent)	1.07 (0.44–2.61)	1.00 (referent)
Model 3*** OR (95% CI)	0.54 (0.14–2.04)	0.98 (0.31–3.07)	0.42 (0.09–1.89)	1.00 (referent)	1.09 (0.45–2.67)	1.00 (referent)

*From conditional logistic regression, controlling for the matching variables only (study centre).
**From conditional logistic regression, controlling for the matching variables (study centre) and maternal age, number of prior preterm deliveries, smoking status, BMI, season when blood drawn, and treatment group.
***From conditional logistic regression, controlling for all variables in model 2 plus fish intake.

US academic health centres should reduce concerns about widespread low vitamin D status in women of reproductive age. Indeed, our median 25(OH)D concentration was quite similar to the mean value from women in a nationwide sample of pregnant women:[2] 67 and 65 nmol/l, respectively. The health benefits and risks of raising 25(OH)D concentrations in pregnant women (e.g. to >100 nmol/l) are not clear, and merit further investigation.[14]

In interpreting our previous finding that fish consumption was protective against recurrent preterm birth,[12] we hypothesised that the protective effects may have been mediated via differences in vitamin D status, as fatty fish and fish liver oils are the main natural dietary sources for vitamin D. Contrary to our expectation, we observed a negative correlation between fish consumption and serum 25(OH)D concentration [i.e. a higher intake of fish was associated with lower concentrations of 25(OH)D]. This would point to other sources of vitamin D being available to this group: i.e. from ultraviolet irradiation of the skin; from fortified foods (such as milk, cereal or orange juice); or from vitamin D supplements. We did not ascertain the usage of prenatal vitamins, and vitamin D levels in prenatal vitamins vary from 200 to 800 IU.

Potential limitations of our report include the following. First, we only measured 25(OH)D at two time points in mid-pregnancy, with the second time point including a significantly smaller number of participants. Multiple measures across pregnancy may have demonstrated different outcomes. Also, and as stated earlier, we studied a group of mothers in which fewer than anticipated had serum 25(OH)D concentrations of <50 nmol/l, and thus our results may not be generalisable to women who enter pregnancy with a low vitamin D status. Strengths include the prospective design, subjects from multiple centres across the USA, and the high prevalence of recurrent preterm birth.

In conclusion, mid-gestation serum 25(OH)D concentrations of <50 nmol/l occurred less often than we anticipated, and was not associated with recurrent preterm birth. Several clinical trials have failed to show any effect of vitamin D supplementation on the risk of preterm birth. These trials have been small, and lacked adequate power to conclusively answer the important question.[12–15] Kovacs et al. have published a comprehensive review article that thoroughly addresses the biology, toxicology, and epidemiology of this important nutrient for pregnancy and lactation[16]. Although the health benefits of vitamin D supplementation remain unclear,[3–9,15] these data do not support its routine use for the prevention of recurrent preterm birth.

Disclosure of interests

None.

Contribution to authorship

All authors listed were significant contributors to the design and implementation of the study. The data were analysed by KM and the lab analysis was performed by CAC. All authors reviewed the article and contributed to the final submission.

Details of ethics approval

The study (NCT00135902 at www.clinicaltrials.gov) was approved by the IRBs of the biostatistical coordinating

centre and all participating clinical centres, and this secondary analysis was determined by the IRB office of the University of North Carolina (Chapel Hill, NC, USA) to be exempt from IRB review of the Office of Human Subjects. All enrolled women gave written informed consent.

Funding

The project described was supported by grants from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) (HD27860, HD27917, HD40560, HD34208, HD40485, HD21410, HD27915, HD 40500, HD40512, HD40544, MO1-RR-000080, HD34136, HD27869, HD40545, HD36801, and HD19897), and does not necessarily represent the official views of the NICHD or National Institutes of Health (NIH). CAC was supported, in part, by the Massachusetts General Hospital Center for D-receptor Activation Research (Boston, MA, USA).

Acknowledgements

The authors thank the following MFMU Network members who participated in protocol development and coordination between clinical research centres (Karen Dorman), protocol/data management and statistical analysis (Madeline Rice and Elizabeth Thom), and protocol development and oversight (Catherine Y. Spong). In addition to the authors, other members of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Maternal–Fetal Medicine Units Network are as follows: K. Dorman, E. Prata, and K. Hamden of University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; P. Meis, M. Swain, B. Scott, and C. Leftwich of Wake Forest University Health Sciences, Winston-Salem, NC, USA; G. Norman, D. Driscoll, C. Sudz, L. Wynn, and S. Blackwell of Wayne State University, Detroit, MI, USA; K. Anderson (University of Utah Health Sciences Center), S. Bonnemort (McKay-Dee Hospital), D. Lund (University of Utah Health Sciences Center), J. Russell (LDS Hospital) and J. Parsons (Utah Valley Regional Medical Center) of University of Utah Health Sciences Center, Salt Lake City, UT, USA; S. Bousleiman, S. South, V. Carmona, H. Husami, C. Lankford, and C. Perez of Columbia University, New York, NY, USA; M. Luce, and M. Cotroneo of University of Pittsburgh, Pittsburgh, PA, USA; F. Johnson, M. Landon, D. Cline, and H. Walker of the Ohio State University, Columbus, OH, USA; D. Allard and J. Tillinghast of the Women and Infants Hospital, Brown University, Providence, RI, USA; M. Dinsmoor (NorthShore University HealthSystem), P.J. Simon, M. Huntley, C. Whitaker-Carr, M. Ramos-Brinson, and G. Mallett of Northwestern University, Chicago, IL, USA; C. Milluzzi, J. Hunter, W. Dalton, H. Ehrenberg, and B. Stetzer of Case Western Reserve University, MetroHealth Medical Center, Cleveland, OH, USA; M. Hoffman, M. Talucci, C. Tocci, S. Wilson, and M. Lake, Drexel University College of Medicine, Philadelphia, PA, USA; W.W. Andrews, A. Northen, M. Parks, P. Blake Files of the University of Alabama at Birmingham, Birmingham, AL, USA; L.C. Gilstrap, B. Glenn-Cole, and K. Cannon of The University of Texas Health Science Center at Houston, Houston, TX, USA; E. Thom, M. Rice, J. Zachary, R. Palugod, and L. Leuchtenburg of The George Washington University Biostatistics Center, Rockville, MD, USA; and C. Spong and S. Tolivaisa of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, USA. ■

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