DOI: 10.1111/j.1471-0528.2012.03307.x
www.bjog.org

Longitudinal vitamin D status in pregnancy and the risk of pre-eclampsia

SQ Wei,[a] F Audibert,[a] N Hidiroglou,[b,*] K Sarafin,[b] P Julien,[c] Y Wu,[a] ZC Luo,[a] WD Fraser[a]

[a] Department of Obstetrics and Gynecology, Sainte-Justine Hospital, University of Montreal, Montreal, QC [b] Bureau of Nutritional Sciences, Health Canada, Ottawa, ON [c] Lipid Research Center, Laval University Medical Center (CHUQ), Québec city, QC, Canada
Correspondence: SQ Wei, Department of Obstetrics and Gynecology, CHU Sainte-Justine, Université de Montréal, 5757 Decelles, Montreal, QC H3S 2C3, Canada. Email: shu.qin.wei@umontreal.ca
*Deceased.

Accepted 6 February 2012. Published Online 29 March 2012.

Objective Whether vitamin D deficiency in pregnancy is a cause of pre-eclampsia remains controversial. Most previous studies to date have assessed exposure at only one time-point in pregnancy. We assessed longitudinal vitamin D status during pregnancy and the risk of pre-eclampsia.

Design Prospective cohort study.

Setting Seventeen urban obstetric hospitals, Canada.

Population Pregnant women who were participants in a trial of vitamin C and E supplementation for the prevention of pre-eclampsia. Canadian participants who consented to participate in a biobank with plasma specimens available at the baseline visit were included (n = 697).

Methods Maternal plasma 25-hydroxyvitamin D (25(OH)D) concentrations were measured at 12–18 and 24–26 weeks of gestation using chemiluminescence immunoassay.

Main outcome measures Pre-eclampsia.

Results Of the women, 39% were vitamin D deficient (25(OH)D <50 nmol/l). A strong positive correlation was observed in maternal 25(OH)D concentrations between the two gestational age windows (r = 0.69, P < 0.0001). Mean maternal 25(OH)D concentrations at 24–26 weeks of gestation were significantly lower in women who subsequently developed pre-eclampsia compared with those who did not (mean ± SD: 48.9 ± 16.8 versus 57.0 ± 19.1 nmol/l, P = 0.03). Women with 25(OH)D < 50 nmol/l at 24–26 weeks gestation experienced an increased risk of pre-eclampsia (adjusted odds ratio 3.24, 95% confidence interval 1.37–7.69), whereas the association was not statistically significant for maternal 25(OH)D level at 12–18 weeks of gestation.

Conclusions Lower maternal 25(OH)D levels at late mid-trimester were associated with an increased risk of pre-eclampsia.

Keywords 25-Hydroxyvitamin D, pre-eclampsia, pregnancy, vitamin D.

Please cite this paper as: Dr Wei SQ, Audibert F, Hidiroglou N, Sarafin K, Julien P, Wu Y, Luo ZC, Fraser WD. Longitudinal vitamin D status in pregnancy and the risk of pre-eclampsia. BJOG 2012;119:832–839.

Introduction

Pre-eclampsia, a pregnancy disorder characterised by hypertension and proteinuria, complicates 2–8% of all pregnancies and accounts for 25% of all maternal deaths.[1,2] Pre-eclampsia remains a leading cause of maternal and perinatal morbidity and mortality.[2,3] The aetiology of pre-eclampsia remains largely unknown. It has been hypothesised that abnormal trophoblast invasion, inflammatory responses, oxidative stress and endothelial dysfunction are all potential contributing factors in this disorder.[4,5]

Maternal vitamin D deficiency is common during pregnancy and a widespread public health problem.[6] A high prevalence of vitamin D deficiency has been observed among pregnant women,[7] with prevalence rates varying by ethnicity[8] and sunlight exposure.[9] There is increasing interest in a range of actions of vitamin D in pregnancy, including its effects on placental function and inflammatory response.[10] Pro-inflammatory cytokines such as tumour necrosis factor-α, interleukin-6 and interferon-γ have been reported to be increased in pregnancies with vitamin D deficiency.[11] Attention has been drawn to vitamin D as a possible aetiological factor in pre-eclampsia. Vitamin D deficiency during pregnancy, based on low circulating 25-hydroxyvitamin D (25(OH)D) level, has been linked to a number of serious health problems including poor bone mineralisation in infants,[12] low birthweight,[13] and other adverse pregnancy outcomes.[14,15] However, except for two

small studies[16,17] (total $n \leq 50$), most studies to date have assessed exposure at only one time-point in pregnancy. It is uncertain whether there is a critical window for exposure during pregnancy that increases the risk of pre-eclampsia. Recent studies[18,19] have underlined the need for prospective longitudinal data on the effect of maternal vitamin D status during pregnancy on the risk of pre-eclampsia. The present study aimed to serially assess maternal vitamin D status during pregnancy and to determine the association between maternal 25(OH)D levels at early and late mid-trimester gestational age windows and the risk of pre-eclampsia.

Methods

Study design

This prospective cohort study was based on a bank of data and biological specimens generated in the context of a randomised, placebo-controlled international trial of antioxidant supplementation (vitamins C and E) for the prevention of pre-eclampsia (International Trial of Antioxidants in the Prevention of Pre-eclampsia, INTAPP). The trial was conducted in Canada (17 centres) and Mexico (ten centres) between January 2004 and March 2006. The design and methods of the trial have been described previously.[20] Canadian INTAPP participants who were re-contacted and consented to participate in a biobank for further research and for whom a maternal plasma specimen was available at the baseline study visit contributed to this study ($n = 697$). The most frequent reason for failure to re-consent Canadian INTAPP participants was inability to contact them. Mexican INTAPP participants did not contribute data to this study because it was not feasible to re-contact them to obtain their consent for use of their biological specimens for this purpose. Maternal characteristics and clinical outcomes were similar and not significantly different among Canadian women who consented to contribute to the biobank and those who did not. Women were stratified in INTAPP according to the presence or absence of risk factors for pre-eclampsia. Women were in the high-risk stratum for pre-eclampsia if they met at least one of the four criteria: pre-pregnancy chronic hypertension (diastolic blood pressure ≥90 mmHg or use of antihypertensive medication before 20 weeks of gestation), prepregnancy diabetes, a multiple pregnancy, or a history of pre-eclampsia.[20] The remaining women were nulliparae without other risk factors for pre-eclampsia (low-risk group). Non-fasting maternal blood samples were collected and banked at 12–18 and 24–26 weeks of gestation. Venous blood was drawn into EDTA tubes and plasma samples were immediately separated by centrifugation at 500 ***g*** for 10 minutes at 4°C. Plasma samples were rapidly frozen and stored at −80°C for future assays. This study had a power of 87% to detect a risk ratio of 2.0 or greater for the association between vitamin D deficiency and pre-eclampsia.

The study definitions of gestational hypertension, pre-eclampsia and severe pre-eclampsia according to the criteria of the Canadian Hypertension Society Consensus Conference Report[21] have been described previously.[20] Briefly, gestational hypertension was defined as two or more readings of diastolic blood pressure ≥90 mmHg taken 4 hours apart but within 72 hours occurring at ≥20 weeks of gestation.[21] Pre-eclampsia was defined as gestational hypertension with proteinuria. Proteinuria was defined as a urine protein dipstick test ≥2+, or the urinary excretion of ≥0.3 g in a 24-hour urine collection.[21] Superimposed pre-eclampsia and severe pre-eclampsia were defined according to the guidelines of Canadian Hypertension Society Consensus Conference Report.[21]

25(OH)D assay

Plasma aliquots for each woman were shipped on dry ice to the Health Canada Nutrition Research Division laboratory (Ottawa, ON, Canada) for plasma 25(OH)D analyses. The Health Canada laboratory has been in proficient standing with the Vitamin D External Quality Assessment Scheme (DEQAS).[22] 25(OH)D was measured by a direct, competitive chemiluminescence immunoassay using the DiaSorin LIAISON 25(OH)D TOTAL assay (DiaSorin, Inc., Stillwater, MN, USA).[23] The assay was performed in accordance with the manufacturer's product insert. The assay uses 25(OH)D conjugated to an isoluminol derivative to compete with the 25(OH)D present in the plasma. Both conjugated and unconjugated 25(OH)D compete for the antibody, which has been coated onto magnetic particles. A starter reagent is added to initiate a flash chemiluminescent reaction of the isoluminol derivative, which is then measured by a photomultiplier. The amount of light measured is inversely proportional to the concentration of 25(OH)D present in a given sample. A manufacturer's predefined master curve is adjusted to an instrument-specific calibration curve using two supplied calibration samples with known 25(OH)D concentrations. The adjusted calibration curve is then used to determine the 25(OH)D concentrations of the unknown samples. The lower and upper limits of detection were 10 nmol/l and 375 nmol/l, respectively. Two levels of quality control provided by the manufacturer (Diasorin Inc.), along with three external quality controls (Biorad Diagnostics Inc., Mississauga, Ontario, Canada) were run in each assay. The intra-assay coefficient of variation was 5.2–9.2%, and the inter-assay coefficient of variation was 7.9–9.2%, respectively.

Statistical analysis

Maternal characteristics of pre-eclamptic and nonpre-eclamptic women were compared using chi-square, Fisher's

exact, Student's *t* or Wilcoxon rank test, where appropriate. Spearman correlation was applied to examine the association in maternal 25(OH)D concentrations between the two gestational age windows (12–18 weeks and 24–26 weeks of gestation). Plasma 25(OH)D concentrations were first investigated as a continuous variable. Risk curve-fitting was applied to assess the potential dose–response relation between 25(OH)D level and the risk of pre-eclampsia.[24] We then conducted logistic regression analyses adjusting for potential confounding factors to estimate the risk (adjusted odds ratio [aOR] with 95% confidence intervals [CI]) of pre-eclampsia in women who had a 25(OH)D level <50 nmol/l versus those who did not. There is no universally accepted definition of maternal vitamin D deficiency. We used the cutoff point of 50 nmol/l, which has been suggested by some experts to define vitamin D deficiency.[25] Subgroup analyses were performed to explore the associations in high-risk and low-risk strata. The available co-variables included vitamin C and E treatment, ethnicity, season when blood was drawn, maternal age, education, marital status, parity, smoking, prepregnancy body mass index (BMI), prenatal vitamin supplementation, and the risk group. Prespecified potential confounders (risk stratum, maternal age, smoking, prepregnancy BMI, season when blood was drawn) and additional co-variables at $P < 0.10$ were included in the final logistic regression models. Two-sided P values < 0.05 were considered statistically significant. All analyses were performed using SAS software, version 9.2 (SAS Institute Inc, Cary, NC, USA).

Results

Of the 697 study participants, 32 (4.6%) developed pre-eclampsia, including 23 severe pre-eclampsia. There were no significant differences between pre-eclamptic and nonpre-eclamptic women in maternal age, education, ethnic origin, marital status, vitamin C and E treatment, prenatal vitamin supplementation, smoking or season when blood was drawn (Table 1). Plasma 25(OH)D concentrations were lower in non-Caucasians than in Caucasians at 12–18 weeks of gestation (43.7 ± 16.0 versus 57.3 ± 18.8 nmol/l, $P < 0.001$) and 24–26 weeks of gestation (43.5 ± 16.0 versus 58.2 ± 18.8 nmol/l, $P < 0.001$). Plasma 25(OH)D concentrations were lower in winter than in non-winter seasons at 12–18 weeks of gestation (52.1 ± 19.5 versus 59.4 ± 17.7 nmol/l, $P < 0.001$) and 24–26 weeks of gestation (52.3 ± 17.7 versus 60.8 ± 19.4 nmol/l, $P < 0.001$). In the high-risk stratum ($n = 229$), 21 women (9.2%) developed pre-eclampsia, whereas in the low-risk group ($n = 468$), 11 women (2.4%) developed pre-eclampsia. Women who subsequently developed pre-eclampsia had a higher mean prepregnancy BMI and higher systolic and diastolic blood pressures at the baseline study visit. Women who developed pre-eclampsia were more likely to deliver early. Plasma 25(OH)D was measured at a mean of 14.6 weeks and 25.0 weeks of gestation at visits 1 and 2, respectively. The plasma samples were drawn at similar gestational ages between pre-eclamptic and nonpre-eclamptic women. Maternal 25(OH)D concentrations in women who developed gestational hypertension only (without proteinuria) ($n = 81$) were similar to those in women who remained normotensive both at visit 1 and 2 (all $P > 0.1$) (data not shown) and were included in the nonpre-eclamptic group.

At 12–18 weeks of gestation, among women who later developed pre-eclampsia, the mean maternal plasma 25(OH)D concentration was lower than in nonpre-eclamptic women, but the difference was not statistically significant (51.1 ± 14.8 nmol/l versus 56.0 ± 19.1 nmol/l, $P = 0.16$); whereas at 24–26 weeks of gestation, maternal 25(OH)D

Table 1. Characteristics of the study population

Characteristics	Pre-eclamptic *n* = 32	Nonpre-eclamptic *n* = 665
Maternal age (years)	30.9 ± 5.3	30.3 ± 4.8
Maternal education (years)	16.2 ± 2.7	16.2 ± 2.8
Ethnicity, Caucasian	27 (84.4)	593 (89.2)
Maternal prepregnancy BMI, ≥30.0 kg/m^2	11 (34.4)*	107(17.2)
Married/Common Law	29 (90.6)	622 (94.5)
Employed	26 (81.3)	567 (85.3)
Smoking	6 (18.8)	144 (21.7)
Nulliparity	15 (44.1)*	542 (81.5)
High-risk group**	21 (65.6)*	208 (31.3)
Mean systolic BP at trial entry (mmHg)	122.9 ± 13.3*	111.6 ± 11.9
Mean diastolic BP at trial entry (mmHg)	75.1 ± 8.9*	67.7 ± 8.6
Vitamin C + E treatment group	17 (53.1)	320 (48.1)
Prenatal vitamin supplementation	31 (96.9)	637 (96.1)
Gestational age at visit 1 (weeks)	14.6 ± 1.6	14.8 ± 2.1
Gestational age at visit 2 (weeks)	25.0 ± 1.3	24.8 ± 2.5
Gestational age at delivery (weeks)	35.8 ± 3.3*	38.3 ± 2.6
Season of blood draw (visit 1)		
Winter (2 November to 30 April)	13 (40.6)	332 (49.9)
Non-winter	19 (59.4)	333 (50.1)
Season of blood draw (visit 2)		
Winter (1 November to 30 April)	10 (35.7)	290 (50.0)
Non-winter	18 (64.3)	290 (50.0)
Season of delivery		
Winter (1 November to 30 April)	15 (46.9)	329 (49.6)
Non-winter	17 (53.1)	335 (50.4)

Data are presented as mean ± SD or *n* (%).
BP, blood pressure; BMI, body mass index.
*$P < 0.05$ comparing pre-eclamptic with nonpre-eclamptic women.
**High-risk group including chronic hypertension, prepregnancy diabetes, multiple pregnancy, or a history of pre-eclampsia.

levels were statistically significantly lower in women who went on to develop pre-eclampsia (48.9 ± 16.8 nmol/l versus 57.0 ± 19.1 nmol/l, $P = 0.03$) (Table 2). Interestingly, the mean 25(OH)D concentrations in women with pre-eclampsia decreased slightly over the two gestational age windows (mean Δ25(OH)D: −0.2 nmol/l), whereas for nonpre-eclamptic women, the mean 25(OH)D concentrations increased over gestational age (mean Δ25(OH)D: 0.9 nmol/l), although the difference was not statistically significant. A strong positive correlation was observed in maternal 25(OH)D concentrations between the two gestational age windows ($r = 0.69$; $P < 0.0001$). Subgroup analyses were performed according to risk group stratum. In the low-risk group (nulliparous women without other risk factors), maternal 25(OH)D levels at 24–26 weeks of gestation were significantly lower in women who developed pre-eclampsia compared with nonpre-eclamptic women (50.8 ± 9.4 versus 58.4 ± 18.0 nmol/l, $P = 0.04$). However, in the high-risk group, the difference did not reach statistical significance (48.0 ± 19.6 versus 54.1 ± 20.9 nmol/l, $P = 0.23$).

There was a potential dose–response association between maternal plasma 25(OH)D concentration at 24–26 weeks of gestation and the risk of pre-eclampsia (Figure 1, $P = 0.03$ for the association in logistic regression; $P = 0.35$ in Hosmer–Lemeshow goodness-of-fit test), whereas the association was not statistically significant for maternal plasma 25(OH)D concentration at 12–18 weeks of gestation ($P = 0.16$ for the association). The risk curve suggested a more substantial elevation in risk of pre-eclampsia at approximately <50 nmol/l in plasma 25(OH)D concentration at 24–26 weeks of gestation.

The prevalence rates of maternal 25(OH)D level <50 nmol/l at 12–18 and 24–26 weeks of gestation were 38.8% and 39.1%, respectively. The risk of pre-eclampsia was not significantly different among those with and without 25(OH)D level <50 nmol/l at 12–18 weeks of gestation (5.5% versus 4.0%, $P = 0.34$), whereas there was a significant increase in the risk of pre-eclampsia among those with 25(OH)D level <50nmol/l compared with those without at 24–26 weeks of gestation (8.1% versus 2.5%, $P = 0.001$) (Table 3).

Adjusting for potential confounders, each SD increase in plasma 25(OH)D level at 12–18 weeks of gestation was associated with a non-significant reduction in odds of developing pre-eclampsia (aOR 0.79, 95% CI 0.52–1.20), whereas each SD increase in plasma 25(OH)D level at 24–26 weeks of gestation was associated with a borderline reduction in the odds of pre-eclampsia (aOR 0.68, 95% CI 0.44–1.05, $P = 0.08$) (Table 4). At 24–26 weeks of gestation, maternal 25(OH)D <50 nmol/l was associated with a 3.2-fold increased risk of pre-eclampsia (aOR 3.24, 95% CI 1.37–7.69), whereas at 12–18 weeks of gestation, the risk increase was not statistically significant (aOR 1.24, 95% CI 0.58–2.67).

Discussion

This is the largest prospective cohort study to date to assess the association between vitamin D deficiency and the risk of pre-eclampsia. The main finding of our study is that maternal 25(OH)D <50 nmol/l at 24–26 weeks of gestation is associated with a significantly increased risk of pre-eclampsia (aOR = 3.2). This association seems stronger for nulliparous women without other risk factors. We did not detect a statistically significant association between maternal plasma 25(OH)D level at 12–18 weeks of gestation and pre-eclampsia.

Two studies have measured longitudinal vitamin D levels in pregnant women who developed pre-eclampsia compared with those who did not; both failed to detect any association between vitamin D level and pre-eclampsia, possibly because of their small sample size (total $n \leq 50$).[16,17] Some studies have measured maternal 25(OH)D level at

Table 2. Maternal plasma vitamin D levels at 12–18 and 24–26 weeks of gestation

25(OH)D concentration (nmol/l)	Pre-eclamptic	Nonpre-eclamptic	*P*-value*
Visit 1 (12–18 weeks)	51.1 (14.8)	56.0 (19.1)	0.16
High-risk group** ($n = 229$)	50.5 (15.2) ($n = 21$)	53.9 (19.8) ($n = 208$)	0.44
Low-risk group** ($n = 468$)	52.4 (14.7) ($n = 11$)	56.9 (18.8) ($n = 457$)	0.42
Visit 2 (24–26 weeks)	48.9 (16.8)*	57.0 (19.1)	**0.03**
High-risk group** ($n = 207$)	48.0 (19.6) ($n = 19$)	54.1 (20.9) ($n = 188$)	0.23
Low-risk group** ($n = 397$)	50.8 (9.4)* ($n = 9$)	58.4 (18.0) ($n = 388$)	**0.04**
Δ25(OH)D (V2–V1)	-0.2 (10.5)	0.9 (14.9)	0.69

Data presented are mean (SD).
*P values in Student's t tests for differences in pre-eclamptic vs nonpre-eclamptic women (data distributions were Normal).
**High-risk group included women with chronic hypertension, prepregnancy diabetes, multiple pregnancy, or a history of pre-eclampsia; low-risk group refers to women who were nulliparae without known risk factors for pre-eclampsia.

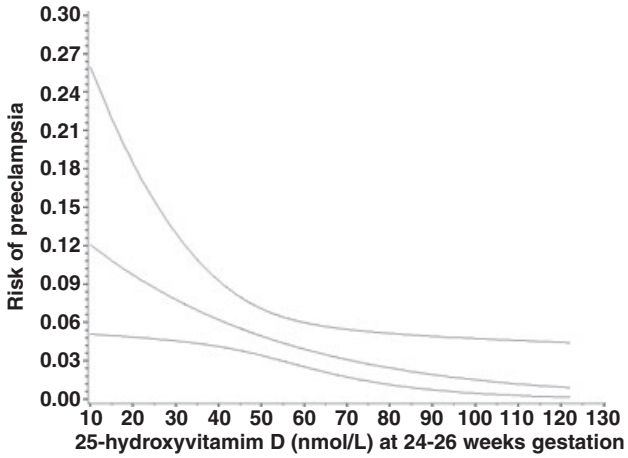

Figure 1. The association between maternal plasma 25-hydroxyvitamin D concentration at 24–26 weeks of gestation and the risk of pre-eclampsia. The predicted probability of pre-eclampsia was derived from an unadjusted logistic regression model ($P = 0.03$). The solid line represents the point estimate, the dotted lines represent the 95% confidence intervals.

Table 3. Risk of pre-eclampsia according to presence or absence of maternal 25(OH)D level <50 nmol/l

Vitamin D status	Pre-eclamptic	Nonpre-eclamptic	*P*-value*
Vitamin D level at 12–18 weeks of gestation (*n* = 697)			
25(OH)D <50 nmol/l (*n* = 272)	15 (5.5)	257 (94.5)	0.34
25(OH)D ≥50 nmol/l (*n* = 425)	17 (4.0)	408 (96.0)	
Vitamin D level at 24–26 weeks of gestation (*n* = 604)			
25(OH)D <50 nmol/l (*n* = 236)	19 (8.1)	217 (91.9)	0.001
25(OH)D ≥50 nmol/l (*n* = 368)	9 (2.5)	359 (97.5)	

Data presented are *n* (%).
**P* values in chi-square tests for differences in rates of pre-eclampsia comparing women with plasma 25(OH)D levels <50 vs ≥50 nmol/l.

one single time-point and the results have been conflicting. Bodnar et al.[26] showed that maternal vitamin D deficiency at <22 weeks of gestation (mean gestational age 10 weeks) was associated with an increased risk of pre-eclampsia. Two studies[18,27] found that maternal vitamin D deficiency was associated with severe pre-eclampsia (in one of these studies,[18] the vitamin D level was measured after the diagnosis of severe pre-eclampsia). In contrast, two other studies[28,29] failed to find an association between maternal vitamin D levels and pre-eclampsia. Shand et al.[28] studied maternal vitamin D status only in a group of high-risk women (mean gestational age 19 weeks) and did not find an association, which is consistent with our results in high-risk women. Caution is warranted in data interpretation because failure to show a significant difference in this subgroup could have been the result of beta-error. Powe et al.[29] studied vitamin D deficiency in the first trimester (mean gestational age 11 weeks) and found no association between maternal 25(OH)D deficiency and the risk of pre-eclampsia. This is consistent with our findings that vitamin D status in early pregnancy (12–18 weeks of gestation) was not significantly associated with pre-eclampsia. Our study showed that low levels of maternal 25(OH)D (<50 nmol/l) at late mid-trimester (24–26 weeks of gestation) but not early pregnancy (12–18 weeks of gestation) were associated with an increased risk of pre-eclampsia. Pre-eclampsia has been hypothesised to be a two-stage disorder. The Two-Stage Model of pre-eclampsia proposes that Stage 1 is the result of failed remodelling of the maternal vessels supplying the intervillous space. This leads to reduced placental perfusion and the release of 'toxins' that result in peripheral endothelial dysfunction and ultimately, the clinical features of pre-eclampsia (Stage 2).[30] Our finding that maternal 25(OH)D level <50 nmol/l at the late second trimester is associated with the risk of pre-eclampsia would suggest that vitamin D may play a role in modulating the peripheral vascular phase of the disease.

Table 4. Unadjusted and adjusted odds ratios of pre-eclampsia in association with maternal plasma 25(OH)D concentration at 12–18 and 24–26 weeks of gestation

Plasma 25(OH)D (nmol/l)	Unadjusted		Adjusted*	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
12–18 weeks of gestation				
25(OH)D, per SD increase	0.76 (0.53–1.11)	0.16	0.79 (0.52–1.20)	0.28
25(OH)D <50 nmol/l	1.41 (0.69–2.88)	0.34	1.24 (0.58–2.67)	0.58
24–26 weeks of gestation				
25(OH)D, per SD increase	0.63 (0.42–0.96)	0.03	0.68 (0.44–1.05)	0.08
25(OH)D <50 nmol/l	3.49 (1.55–7.86)	0.003	3.24 (1.37–7.69)	0.008

*Adjusted for risk group (high vs low, see Methods), maternal age, smoking, prepregnancy body mass index and season of blood draw.

The hypothesis that maternal vitamin D status could alter the risk of pre-eclampsia is biologically plausible. 25(OH)D deficiency was reported to be associated with inflammation-linked vascular endothelial dysfunction.[31] The molecular mechanisms linking vitamin D deficiency to endothelial dysfunction might be mediated, in part, by proinflammatory transcription factor nuclear factor-κB (NF-κB), a major proinflammatory nuclear transcription factor, and interleukin-6, reduced vitamin D receptor and 1α-hydroxylase,[32] or lower calcium levels because vitamin D is essential for calcium absorption. The vascular endothelial cell expression of NF-κB and proinflammatory cytokine interleukin-6 and downstream target of NF-κB, were greater in vitamin D-deficient compared with vitamin D-sufficient adults. Interleukin-6 expression in endothelial cells was strongly inversely correlated to 25(OH)D.[31] Concentrations of other circulating proinflammatory cytokines, such as tumour necrosis factor-α[33]and C-reactive protein,[34] are negatively correlated with 25(OH)D concentrations. Oxidative stress is increased in vitamin D-deficient people, an effect that is reversible with replacement.[35] High levels of thiobarbituric-acid-reactive substances, which indicate lipid peroxidation, have been reported in women with vitamin D deficiency.[35] Maternal vitamin D deficiency may predispose to a proinflammatory response, increase oxidative stress and lead to endothelial dysfunction that characterises pre-eclampsia. In addition, there is evidence showing that vitamin D affects the genes responsible for trophoblast invasion and angiogenesis critical for implantation,[36] which might be an important factor in the pathophysiology of pre-eclampsia.

Several studies have reported on the effects of vitamin D supplementation and the risk of pre-eclampsia. Marya et al.[37] conducted a randomised controlled trial of calcium and vitamin D (1200 IU/day) supplementation in 400 women at 20–24 weeks of gestation. Although calcium and vitamin D supplementation was associated with a significant reduction in blood pressure, the intervention did not produce an isolated effect of the vitamin D supplementation on the incidence of pre-eclampsia. Haugen et al.[38] conducted a prospective cohort study in 23 423 nulliparous pregnant women in Norway and observed a 27% reduction in risk of pre-eclampsia in women who reported taking 10–15 μg/day of vitamin D compared with women taking no supplements (OR = 0.73, 95% CI 0.58–0.92). [Correction added after online publication 2 May 2012: 10–15 mg/day has been changed to μg/day]. However, because vitamin D intake is highly correlated with the intake of long-chain n-3 fatty acids in the Norwegian diet, it was not possible to distinguish the separate effects of vitamin D from other dietary components.

Our study has several methodological strengths. First, it included longitudinal vitamin D data at two gestational age windows. Second, it explored maternal vitamin D status in both high-risk and low-risk women, which reflects the real scenario in most obstetric settings. Third, maternal plasma specimens were collected before the clinical manifestations of pre-eclampsia were evident. Fourth, the DiaSorin LIAISON 25 OH Vitamin D assay is an accurate and precise automated tool for 25(OH)D determination.[23]

A limitation of our study was the lack of data on calcium status. The vitamin D endocrine system is pivotal for calcium homeostasis and the principal physiological function recognised for vitamin D is enhancing calcium absorption[10] and calcium supplementation has been associated with a reduced risk of pre-eclampsia (relative risk 0.45, 95% CI 0.31–0.65).[39] However, a recent study has questioned the association between 25(OH)D concentrations and calcium absorption efficiency.[40] In addition, we had no data on vitamin D binding protein (VDBP), which serves as the major binding protein for 25(OH)D. Alterations in VDBP levels in pre-eclamptic pregnancy might account for lower levels of vitamin D. However, a recent study has shown that VDBP at the first trimester is not associated with subsequent pre-eclampsia risk.[29] The ethnicity of most study participants was Caucasian. These results can be generalised only to similar populations. We have no information as to what dose of vitamin D-containing multivitamins supplements the women had taken. However, our plasma biomarker data on maternal vitamin D status should be a much more reliable indicator than questionnaire-based data on maternal vitamin D supplements.

Conclusions

Maternal 25(OH)D level <50 nmol/l at late mid-trimester of pregnancy is associated with an increased risk of pre-eclampsia. Given the high prevalence of such low levels of vitamin D in pregnant women, it could be a modifiable risk factor with important public-health implications. Further confirmation of this finding in other independent large cohorts and additional research on the underlying biological mechanisms should be undertaken before implementing large double-blind randomised trials of vitamin D supplementation for the prevention of pre-eclampsia.

Disclosure of interests

None.

Contribution to authorship

All authors have fulfilled the conditions required for authorship. SQW, WF, ZCL, NH and PJ conceived and designed the project. SQW, KS and YW analysed and interpreted the data. All authors have either drafted the manuscript or critically reviewed the manuscript for important

intellectual content, and have approved the final version of the manuscript.

Details of ethics approval

The study was approved by the Research Ethics Committee of Sainte-Justine hospital on 4 August 2010 (project No. 3075).

Funding

This work was supported by a research grant from the Canadian Institutes of Health Research (CIHR grant No. 78879). SQW was supported by a Scholarship from the CIHR Strategic Training Initiative in Research in Reproductive Health Sciences (STIRRHS), FA by a CIHR New Investigator award, ZCL by a Junior Scholar award from the Fonds de Recherche en Sante du Quebec (FRSQ) and a CIHR New Investigator award, and WDF by a CIHR Canada Research Chair award.

Acknowledgements

We would like to thank the INTAPP Study group[20] and nursing and medical staff at all participating hospitals. ■

References

1 Steegers EA, Von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet* 2010;376:631–44.

2 Villar K, Say L, Gülmezoglu AM, Merialdi M, Lindheimer MD, Betran AP, et al. Eclampsia and pre-eclampsia: a health problem for 2000 years. In: Critchley H, MacLean AB, Poston L, Walker JJ, editors. *Pre-eclampsia*. London: RCOG Press; 2003. pp. 189–207.

3 Shah DM. Pre-eclampsia: new insights. *Curr Opin Nephrol Hypertens* 2007;16:213–20.

4 Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet* 2005; 365:785–99.

5 James JL, Whitley GS, Cartwright JE. Pre-eclampsia: fitting together the placental, immune and cardiovascular pieces. *J Pathol* 2010; 221:363–78.

6 Javaid MK, Crozier SR, Harvey NC, Gale CR, Dennison EM, Boucher BJ, et al. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. *Lancet* 2006; 367:36–43.

7 Kazemi A, Sharifi F, Jafari N, Mousavinasab N. High prevalence of vitamin D deficiency among pregnant women and their newborns in an Iranian population. *J Womens Health (Larchmt)* 2009;18: 835–9.

8 Bodnar LM, Simhan HN. Vitamin D may be a link to black–white disparities in adverse birth outcomes. *Obstet Gynecol Surv* 2010; 65:273–84.

9 Ustuner I, Keskin HL, Tas EE, Neselioglu S, Sengul O, Avsar AF. Maternal serum 25(OH)D levels in the third trimester of pregnancy during the winter season. *J Matern Fetal Neonatal Med* 2011; 24:1421–6.

10 Shin JS, Choi MY, Longtine MS, Nelson DM. Vitamin D effects on pregnancy and the placenta. *Placenta* 2010;31:1027–34.

11 Díaz L, Noyola-Martínez N, Barrera D, Hernández G, Avila E, Halhali A, et al. Calcitriol inhibits TNF-alpha-induced inflammatory cytokines in human trophoblasts. *J Reprod Immunol* 2009;81:17–24.

12 Kogawa M, Findlay DM, Anderson PH, Ormsby R, Vincent C, Morris HA, et al. Osteoclastic metabolism of 25(OH)-vitamin D3: a potential mechanism for optimization of bone resorption. *Endocrinology* 2010;151:4613–25.

13 Bodnar LM, Catov JM, Zmuda JM, Cooper ME, Parrott MS, Roberts JM, et al. Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. *J Nutr* 2010;140:999–1006.

14 Dror DK, Allen LH. Vitamin D inadequacy in pregnancy: biology, outcomes, and interventions. *Nutr Rev* 2010;68:465–77.

15 Lapillonne A. Vitamin D deficiency during pregnancy may impair maternal and fetal outcomes. *Med Hypotheses* 2010;74:71–5.

16 Halhali A, Villa AR, Madrazo E, Soria MC, Mercado E, Díaz L, et al. Longitudinal changes in maternal serum 1,25-dihydroxyvitamin D and insulin like growth factor I levels in pregnant women who developed preeclampsia: comparison with normotensive pregnant women. *J Steroid Biochem Mol Biol* 2004;90:553–6.

17 Azar M, Basu A, Jenkins AJ, Nankervis AJ, Hanssen KF, Scholz H, et al. Serum carotenoids and fat-soluble vitamins in women with type 1 diabetes and preeclampsia: a longitudinal study. *Diabetes Care* 2011;34:1258–64.

18 Robinson CJ, Alanis MC, Wagner CL, Hollis BW, Johnson DD. Plasma 25-hydroxyvitamin D levels in early-onset severe pre-eclampsia. *Am J Obstet Gynecol* 2010;203:366.

19 Robinson CJ, Wagner CL, Hollis BW, Baatz JE, Johnson DD. Maternal vitamin D and fetal growth in early-onset severe pre-eclampsia. *Am J Obstet Gynecol* 2011;204:556.

20 Xu H, Perez-Cuevas R, Xiong X, Reyes H, Roy C, Julien P, et al. An international trial of antioxidants in the prevention of pre-eclampsia (INTAPP). *Am J Obstet Gynecol* 2010;202:239.

21 Helewa ME, Burrows RF, Smith J, Williams K, Brain P, Rabkin SW. Report of the Canadian Hypertension Society consensus conference, 1: definitions, evaluation and classification of hypertensive disorders in pregnancy. *CMAJ* 1997;157:715–25.

22 Carter GD, Berry JL, Gunter E, Jones G, Jones JC, Makin HL, et al. Proficiency testing of 25-hydroxyvitamin D (25-OHD) assays. *J Steroid Biochem Mol Biol* 2010;121:176–9.

23 Wagner D, Hanwell HE, Vieth R. An evaluation of automated methods for measurement of serum 25-hydroxyvitamin D. *Clin Biochem* 2009;42:1549–56.

24 Witte JS, Greenland S. A nested approach to evaluating dose–response and trend. *Ann Epidemiol* 1997;7:188–93.

25 Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266–81.

26 Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. Maternal vitamin D deficiency increases the risk of pre-eclampsia. *J Clin Endocrinol Metab* 2007;92:3517–22.

27 Baker AM, Haeri S, Camargo CA Jr, Espinola JA, Stuebe AM. A nested case–control study of midgestation vitamin D deficiency and risk of severe pre-eclampsia. *J Clin Endocrinol Metab* 2010;95:5105–9.

28 Shand A, Nassar N, Von Dadelszen P, Innis S, Green T. Maternal vitamin D status in pregnancy and adverse pregnancy outcomes in a group at high risk for pre-eclampsia. *Br J Obstet Gynaecol* 2010; 117:1593–8.

29 Powe CE, Seely EW, Rana S, Bhan I, Ecker J, Karumanchi SA, et al. First trimester vitamin D, vitamin D binding protein, and subsequent pre-eclampsia. *Hypertension* 2010;56:758–63.

30 Roberts J. Pre-eclampsia a two-stage disorder: what is the linkage? Are there directed fetal/placental signals? In: Lyall F, Belfort M. *Pre-eclampsia: Etiology and Clinical Practice*. New York: Cambridge University Press; 2007. pp. 183–94.

31 Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-hydroxyvitamin D deficiency is associated with inflammation-linked

vascular endothelial dysfunction in middle-aged and older adults. *Hypertension* 2011;57:63–9.

32 Hoeck AD, Pall ML. Will vitamin D supplementation ameliorate diseases characterized by chronic inflammation and fatigue? *Med Hypotheses* 2011;76:208–13.

33 Peterson CA, Heffernan ME. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. *J Inflamm (Lond)* 2008;5:10.

34 Ngo DT, Sverdlov AL, McNeil JJ, Horowitz JD. Does vitamin D modulate asymmetric dimethylarginine and C-reactive protein concentrations? *Am J Med* 2010;123:335–41.

35 Tarcin O, Yavuz DG, Ozben B, Telli A, Ogunc AV, Yuksel M, et al. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. *J Clin Endocrinol Metab* 2009; 94:4023–30.

36 Evans KN, Bulmer JN, Kilby MD, Hewison M. Vitamin D and placental-decidual function. *J Soc Gynecol Investig* 2004;11:263–71.

37 Marya RK, Rathee S, Manrow M. Effect of calcium and vitamin D supplementation on toxaemia of pregnancy. *Gynecol Obstet Invest* 1987;24:38–42.

38 Haugen M, Brantsaeter AL, Trogstad L, Alexander J, Roth C, Magnus P, et al. Vitamin D supplementation and reduced risk of pre-eclampsia in nulliparous women. *Epidemiology* 2009;20:720–6.

39 Hofmeyr GJ, Lawrie TA, Atallah AN, Duley L. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. *Cochrane Database Syst Rev* 2010;(8): CD001059.

40 Aloia JF, Chen DG, Yeh JK, Chen H. Serum vitamin D metabolites and intestinal calcium absorption efficiency in women. *Am J Clin Nutr* 2010;92:835–40.

