Vitamin D status is associated with uteroplacental dysfunction indicated by pre-eclampsia and small-for-gestational-age birth in a large prospective pregnancy cohort in Ireland with low vitamin D status

Mairead E. Kiely, Joy Y. Zhang, Michael Kinsella, Ali S. Khashan, and Louise C. Kenny

ABSTRACT

Background: Associations between vitamin D and pregnancy outcomes have been inconsistent.

Objectives: We described the distribution of 25-hydroxyvitamin D3 [25(OH)D3], 3-epi-25(OH)D3, and 25(OH)D2 in early pregnancy and investigated associations with pre-eclampsia and small-for-gestational-age (SGA) birth, which are indicative of uteroplacental dysfunction.

Design: The SCOPE (Screening for Pregnancy Endpoints) Ireland prospective pregnancy cohort study included 1768 well-characterized low-risk, nulliparous women resident at 52°N. Serum 25(OH)D3, 3-epi-25(OH)D3, and 25(OH)D2 were quantified at 15 wk of gestation with the use of a CDC-accredited liquid chromatography–tandem mass spectrometry method.

Results: The mean ± SD total 25(OH)D concentration was 56.7 ± 25.9 nmol/L, and 17% and 44% of women had 25(OH)D concentrations <30 and <50 nmol/L, respectively. The prevalence of pre-eclampsia was 3.8%, and 10.7% of infants were SGA. There was a lower risk of pre-eclampsia plus SGA combined (13.6%) at 25(OH)D concentrations >75 nmol/L (adjusted OR: 0.64; 95% CI: 0.43, 0.96). The main predictors of 25(OH)D were the use of vitamin D–containing supplements (adjusted mean difference: 20.1 nmol/L; 95% CI: 18.5, 22.7 nmol/L) and summer sampling (adjusted mean difference: 15.5 nmol/L; 95% CI: 13.4, 17.6 nmol/L). Non-Caucasian ethnicity (adjusted mean difference: −19.3 nmol/L; 95% CI: −25.4, −13.2 nmol/L) and smoking (adjusted mean difference: −7.0 nmol/L; 95% CI: −10.5, −3.6 nmol/L) were negative predictors of 25(OH)D. The mean ± SD concentration of 3-epi-25(OH)D3, which was detectable in 99.9% of samples, was 2.6 ± 1.6 nmol/L. Determinants of 3-epi-25(OH)D3 were 25(OH)D3 (adjusted mean difference: 0.052 nmol/L; 95% CI: 0.050, 0.053 nmol/L) and maternal age (adjusted mean difference: −0.018 nmol/L; 95% CI: −0.026, −0.009 nmol/L). The mean ± SD concentration of 25(OH)D2 was 3.1 ± 2.7 nmol/L, which was present in all samples. No adverse effects of 25(OH)D concentrations >125 nmol/L were observed.

Conclusions: In the first report to our knowledge of CDC-accredited 25(OH)D data and pregnancy outcomes from a large, clinically validated, prospective cohort study, we observed a protective association of a 25(OH)D concentration >75 nmol/L and a reduced risk of uteroplacental dysfunction as indicated by a composite outcome of SGA and pre-eclampsia. Well-designed, adequately powered randomized controlled trials are required to verify this observation. The SCOPE pregnancy cohort was registered at www.anzctr.org.au as ACTRN12607000551493. Am J Clin Nutr doi: 10.3945/ajcn.116.130419.

Keywords: pre-eclampsia, pregnancy, small for gestational age, vitamin D, 25-hydroxyvitamin D

INTRODUCTION

Because fetal and neonatal circulating 25-hydroxyvitamin D [25(OH)D]7 concentrations are dependent on maternal status, it is important to prevent vitamin D deficiency during pregnancy (1). However, vitamin D deficiency has been reported extensively in gravidae from ethnic minority groups (2–5), and low 25(OH)D concentrations are also common in white women during pregnancy (5–9). This deficiency has been associated with adverse perinatal outcomes including pre-eclampsia (10), gestational diabetes (11), and low birth weight (12), although the data have been inconsistent. The

1 Supported by funding from the Irish Government Department of Agriculture (to MEK) through the Food Institutional Research Measure (for JYZ) and The Higher Education Authority Program for Research in Third Level Institutions, Cycle 4 (to MK). MEK is the co-coordinator of the Integrated Project ODIN (Food-based solutions for optimal vitamin D nutrition and health through the life cycle; http://www.odin-vitd.eu), which is funded by the European Commission (grant 613977). LCK is a Science Foundation Ireland principal investigator (grant 08/N.1/B2083). LCK and MEK are principal investigators in the Science Foundation Ireland funded The Irish Centre for Fetal and Neonatal Translational Research (grant 12/RC/2272).

2 Supplemental Figure 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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Institute of Medicine defines a serum 25(OH)D concentration <30 nmol/L as the concentration at which there is increased risk of vitamin D deficiency, 40 nmol/L as the adequacy threshold, and ≥50 nmol/L as vitamin D sufficiency (13) on the basis of skeletal health outcomes. Although the total amount of most nutrients in circulation increases during pregnancy, hemo-dilution may cause concentrations to decrease from ~ 10 wk of gestation (14). The appropriateness of comparing 25(OH)D concentrations in pregnant women with thresholds established in nonpregnant adults is questionable (1), and it is possible that pregnancy-specific cutoffs of 25(OH)D concentration are required (15, 16).

Vitamin D metabolites, such as 3-epi-25-hydroxyvitamin D3 [3-epi-25(OH)D3] and 25(OH)D2, are of interest because there have been few clinical data. Data from in vitro studies have described the C-3 epimerization of 25(OH)D3 and the subsequent hydroxylation of 3-epi-25(OH)D3, showing that C-3 epimerization may be a common metabolic pathway for vitamin D3 metabolites in selected cells independent of cytochrome P-450 enzymes (17–19). Despite having shown the in vitro biological activity, the clinical significance of 3-epi-25(OH)D3 is still unknown, and 3-epi-25(OH)D3 is not included in the calculation of total 25(OH)D (20, 21). However, 3-epi-25(OH)D3 is present in most individuals at low circulating concentrations and in a relatively higher proportion to total 25(OH)D in infants (22–24). On the basis of a recent systematic review, Bailey et al. (21) recommended that more clinical studies are required that describe the relation between serum 25(OH)D3 and 3-epi-25(OH)D3. We recently reported determinants of 3-epi-25(OH)D3 in a large, representative sample of Irish adults (25). Differences in the distributions and concentrations of 3-epi-25(OH)D3 from a previous report in a smaller sample (26) were likely due to differences in the analytic methods and characterization of participants. Currently, there are limited data on 3-epi-25(OH)D3 in small samples of pregnant women (27, 28).

Similarly, data on circulating 25(OH)D2 during pregnancy are scarce. Although the metabolisms of vitamin D2 and vitamin D3 in the human body are similar (29), their equivalency has been debated (30). Two systematic reviews (31, 32) reported that vitamin D2 is more effective in raising serum 25(OH)D concentrations than is vitamin D2; this finding is potentially important in populations that may be vulnerable to vitamin D deficiency such as pregnant women. Thus, the aims of the current study were to describe the concentrations of 25(OH)D3, 3-epi-25(OH)D3, and 25(OH)D2 in a large, well-phenotyped, prospective pregnancy cohort who were resident at 52°N and to investigate associations between vitamin D status and pre-eclampsia and small-for-gestational-age (SGA) birth, which are indicative of uteroplacental dysfunction.

METHODS

Study design

A total of 1768 participants who attended for antenatal care at Cork University Maternity Hospital, Cork, Ireland (52°N), were recruited to SCOPE (Screening for Pregnancy Endpoints) Ireland study (www.anzctr.org.au; ACTRN12607000551493) before 15 wk of gestation between March 2008 and January 2011 (the participant flowchart is shown in Supplemental Figure 1).

The SCOPE study is an international pregnancy cohort study with the primary aim of developing screening tests for pre-eclampsia, SGA, and spontaneous preterm birth. Six research centers in Auckland, Adelaide, London, Leeds, Manchester, and Cork participated in the study. Data collected in Ireland are reported in the current study. The SCOPE study was conducted according to the Declaration of Helsinki guidelines, and the Clinical Research Ethics Committee of the Cork Teaching Hospitals approved all procedures [ECM5(10)05/02/08].

Complete details of the clinical study and data collection have been provided elsewhere (33, 34). Briefly, the main inclusion criteria were a low-risk pregnancy, a singleton pregnancy ≤ 15 wk of gestation, and no previous pregnancy > 20 wk of gestation. Specific exclusion criteria were as follows: predetermined high risk of pre-eclampsia; an SGA baby; or spontaneous preterm birth because of underlying medical conditions including chronic hypertension requiring antihypertensive drugs, diabetes, renal disease, systemic lupus erythematosus, antiphospholipid syndrome, sickle cell disease, HIV, a previous cervical knife cone biopsy, ≥ 3 terminations of a pregnancy, ≥ 3 miscarriages, or current ruptured membranes; known major fetal anomaly or abnormal karyotype; or an intervention that could modify the outcome of pregnancy (such as aspirin use or cervical cerclage).

All women provided written informed consent and had their first SCOPE visit at 15 wk of gestation (range: 14–16 wk of gestation). Research midwives interviewed participants and collected information on socioeconomic status, occupation, educational attainment, marital or relationship status, and a complete medical history. Data on nutritional supplement use, including brand names, alcohol consumption, smoking history, and recreational drug use, were recorded for the period before conception, during the first trimester, and at the time of the 15-wk visit. Habitual occupational and recreational physical activity was investigated with the use of a standard questionnaire that recorded the frequency and time spent in light and moderate or vigorous activities over a typical month. Vigorous exercise was defined as daily exercise leading to heavy breathing or being out of breath (35). Maternal anthropometric measurements [height and weight for the calculation of BMI (in kg/m2)] were carried out, blood pressure (BP) (2 consecutive measurements taken with the use of a mercury or aneroid sphygmomanometer) was measured, proteinuria was assessed with the use of a semiquantitative, automated dip-stick reading, and a non-fasting blood sample was obtained from each participant. Blood was processed within 3 h to serum and stored at −80°C for future analysis.

Clinical characterization of pregnancy outcomes

Participants were followed prospectively, and pregnancy-outcome data and neonatal measurements were classified according to predefined protocols. Pre-eclampsia was defined as having systolic BP ≥ 140 mm Hg or diastolic BP ≥ 90 mm Hg on ≥ 2 occasions 4 h apart after 20 wk of gestation but before the onset of labor or postpartum with either proteinuria (24-h urinary protein ≥ 300 mg or a spot urine protein:creatinine ratio ≥ 30 mg/mmol creatinine or urine dipstick protein ≥ 2) or any multisystem complication of pre-eclampsia (33). Secondary diagnoses of pre-eclampsia were term pre-eclampsia (gestation at delivery ≥ 37 wk), preterm pre-eclampsia (gestation at delivery
<37 wk), and early-onset pre-eclampsia (gestation at delivery <34 wk). SGA was defined as a birth weight <10th of the customized percentile adjusted for maternal height, booking weight, ethnicity, delivery gestation, and infant sex. The combined prevalence of pre-eclampsia and SGA was indicative of uteroplacental dysfunction. Research midwives entered all data into a certified, secure, Internet-deployed, custom database (MedSciNet AB). Data were extensively monitored throughout the study.

**Analytic methods**

Circulating 25(OH)D₃, 3-epi-25(OH)D₃, and 25(OH)D₂ concentrations were measured at the Cork Centre for Vitamin D and Nutrition Research laboratory with the use of a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method, which has been fully described earlier (25, 34, 35). The instrument used was a Waters Acquity UPLC system coupled to an Acquity Triple Quadrupole TQD mass-spectrometer detector (Waters). Concentrations of 25(OH)D₃ and 25(OH)D₂ were quantified individually, and their values were summed to generate total 25(OH)D. Chromatographic separation and quantitation of 3-epi-25(OH)D₂ were also achieved. Four amounts of serum-based National Institute of Standards and Technology–certified quality-assurance material (standard reference material 972) were used for method validation, whereas quality-control materials that were assayed in parallel to all samples were purchased from Chromsystems. National Institute of Standards and Technology calibrators were used throughout the analysis (standard reference material 2972). The interassay CV was <5% for all metabolites, and the intra-assay CV was <6%. The limits of detection for 25(OH)D₃, 3-epi-25(OH)D₃, and 25(OH)D₂ were 0.31, 0.20, and 0.44 nmol/L, respectively. The limit of quantitation was 1.03, 0.66, and 1.43 nmol/L, respectively. Our method did not measure the C-3 epimer of 25(OH)D₂, but because of the typically low concentrations of 25(OH)D₂ in human sera [current median: 2.6; IQR: 1.7–3.8 nmol/L], we expected the concentrations of 3-epi-25(OH)D₂ to be extremely low. The quality and accuracy of the total 25(OH)D analysis in our laboratory with the use of the LC-MS/MS method was assessed on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (Charing Cross Hospital). This scheme does not delineate 25(OH)D₃ and 25(OH)D₂ or their respective C-3 epimers. We participate in the CDC Vitamin D Standardization Certification program, which reports the accuracy and bias for total 25(OH)D, 25(OH)D₃, 3-epi-25(OH)D₃, and 25(OH)D₂. We are partners in the Vitamin D Standardization Program initiative (36); data describing the excellent performance of our vitamin D analytic method in this project have been provided previously (25, 37, 38).

**Statistical analysis**

The statistical analysis was conducted with the use of PASW Statistics version 20.0 software (SPSS, IBM) and STATA 13.0 (StataCorp LP) software. Descriptive statistics (mean ± SD; median and IQR; and frequency and percentage) were determined for all variables. Seasonal differences were tested with the use of the Mann-Whitney U test for independent samples. Cutoffs for serum 25(OH)D that were proposed by the Institute of Medicine (13) and in the vitamin D literature were used to describe the prevalence of 25(OH)D below various thresholds. Multiple linear regression was used to explore the predictors for concentrations of 25(OH)D and 3-epi-25(OH)D₃ at 15 wk of gestation. Determinants of total 25(OH)D were explored in unadjusted models, and determinants that had a P value ≤0.1 were included in the multivariate model. The residuals of the multiple linear regression model were slightly deviated from the normal distribution; therefore, square-root transformation for the 25(OH)D variable was used, and the residuals were approximately normally distributed when the regression model was repeated. However, because the regression models on the original scale and the square-root transformation scale identified the same factors as significantly associated with 25(OH)D, we report the results from the linear regression model without transformation. The robust option in the STATA program was used with the linear regression models to ensure robust 95% CIs. All tests were 2-sided at the 5% statistical significance level. To assess the association between 25(OH)D₃ and 3-epi-25(OH)D₃, we fitted a fractional polynomial linear regression model with 3-epi-25(OH)D₃ as the outcome and with 25(OH)D₃ as the predictor. We performed crude and adjusted logistic regression models to examine the association between total 25(OH)D and 25(OH)D₃ and risks of pre-eclampsia and SGA birth and the composite outcome of pre-eclampsia and SGA as indicative of uteroplacental dysfunction. The models were adjusted for data collected at the 15-wk visit, including vitamin supplementation, the season of the blood sample, the number of summer days during pregnancy, recreational walking, job status, socioeconomic index, maternal age, BMI, smoking, alcohol use, education, ethnic origin, and marital status.

**RESULTS**

**Sample description**

Characteristics of the cohort of 1768 low-risk primiparous women at 15 wk of gestation are presented in Table 1. The mean ± SD maternal age was 30.5 ± 4.5 y, mean ± SD BMI was 24.9 ± 4.2, mean systolic BP was 105 mm Hg (10), and mean diastolic BP was 66 mm Hg (7). The cohort was primarily white skinned, and the 41 women of non-European origin were Chinese, South Asian, and African. Most women were married or in a stable relationship, and ~50% of women had a tertiary education. Of the 40% of women who were taking vitamin D–containing supplements, 82% consumed 5 μg/d. Doses ranged from 2.5 to 10 μg/d, and almost all products (97%) contained vitamin D₃.

**Vitamin D status**

Cohort concentrations of total 25(OH)D, 25(OH)D₃, 3-epi-25(OH)D₃, and 25(OH)D₂ stratified by winter (November through May) and summer (June through October) seasons are presented in Table 2. As expected, 25(OH)D₃ contributed 93% of total 25(OH)D concentrations, and 3-epi-25(OH)D₃ was detectable in all samples with the exception of 2 participants who had 25(OH)D₃ concentrations <15 nmol/L. The median molar ratio of 25(OH)D₃ to 3-epi-25(OH)D₃ was 21.3 (IQR: 18.6, 24.9). The mean percentage of 3-epi-25(OH)D₃ over 25(OH)D₃
Very few women in the cohort (0.5%) had 25(OH)D concentrations >125 nmol/L.

Effect modifiers of serum 25(OH)D concentrations expressed as adjusted mean differences (95% CIs) in nmol/L are shown in Table 4. Other than the pronounced positive effect of summer and vitamin D supplement use, physical activity was associated with increased 25(OH)D, although there was an inconsistency with respect to vigorous activity, which may have been outdoor or gym oriented. Serum 25(OH)D was higher in participants who walked recreationally ≥4 times/wk. The negative effect of nonwhite ethnicity on 25(OH)D, particularly in the winter (adjusted mean difference: −25.9 nmol/L; 95% CI: −33.5, −18.3 nmol/L) was pronounced. Smoking during, but not before, pregnancy (10% prevalence) had a negative effect on 25(OH)D as well as increasing BMI at 15 wk of gestation.

Pregnancy outcome

The prevalence of pre-eclampsia was 3.8% (70% were term pre-eclampsia cases), and 10.7% of infants were SGA births with a combined prevalence of 13.6%. The association of total 25(OH)D with pre-eclampsia, SGA, and pre-eclampsia plus SGA as a composite indicator of uteroplacental dysfunction is presented in Table 5. With a range of 50–75 nmol/L as a reference threshold, we observed a protective effect (adjusted OR: 0.64; 95% CI: 0.43, 0.96) of having a 25(OH)D concentration >75 nmol/L on the composite pre-eclampsia plus SGA outcome. This association was robust after adjustment for all of the determinants of 25(OH)D that are listed in Table 4 as well as for additional sociodemographic characteristics that are known to influence the pregnancy outcome. There was no adverse effect of high 25(OH)D, although the number of women with concentrations >125 nmol/L was small (n = 8).

TABLE 2
Concentrations of vitamin D metabolites in the SCOPE Ireland pregnancy cohort at 15 wk of gestation stratified by winter (November through May) and summer (June through October) seasons

<table>
<thead>
<tr>
<th>Vitamin D Metabolite</th>
<th>Winter (n = 1034), 2 nmol/L</th>
<th>Summer (n = 734), 2 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total 25(OH)D</td>
<td>49.6 ± 24.6 (45.3–30.2–64.6)</td>
<td>66.7 ± 24.3 (66.4–49.3–83.2)</td>
</tr>
<tr>
<td>25(OH)D1</td>
<td>46.3 ± 24.6 (42.0–26.4–63.3)</td>
<td>63.7 ± 24.5 (63.4–46.3–80.1)</td>
</tr>
<tr>
<td>3-epi-25(OH)D1</td>
<td>2.1 ± 1.3 (1.8–12.2–2.8)</td>
<td>3.3 ± 1.6 (3.1–2.2–4.2)</td>
</tr>
<tr>
<td>25(OH)D2</td>
<td>3.1 ± 1.6 (2.7–1.9–4.0)</td>
<td>2.9 ± 2.9 (2.3–1.6–3.5)</td>
</tr>
</tbody>
</table>

1SCOPE, Screening for Pregnancy Endpoints; 25(OH)D, 25-hydroxyvitamin D; 3-epi-25(OH)D1, 3-epi-25-hydroxyvitamin D1.
2Independent-samples Mann-Whitney U test was used to test seasonal differences. Total 25(OH)D, 25(OH)D1, and 3-epi-25(OH)D1 were all higher in the summer (P < 0.001), and 25(OH)D2 was higher in the winter (P < 0.001).
VITAMIN D AND PREGNANCY OUTCOME

DISCUSSION

To our knowledge, this is the first report of serum concentrations of 25(OH)D analyzed with the use of a CDC-accredited LC-MS/MS method in a large prospective pregnancy cohort with clinically validated pregnancy outcomes. We observed an independent protective association between serum 25(OH)D concentrations >75 nmol/L and reduced risk of uteroplacental dysfunction as indicated by a composite pre-eclampsia plus SGA outcome. This is one of the largest population studies, and the largest study in pregnant women, to describe the distribution of 3-epi-25(OH)D₃, which was detectable in every participant with a serum 25(OH)D concentration >15 nmol/L and was almost entirely attributable to circulating 25(OH)D₃. Another novel aspect of this study was the description of circulating 25(OH)D₂, which was present in almost all women, although vitamin D₂ was consumed in supplement form by a small minority.

Our estimates of 17% and 44% of women with 25(OH)D concentrations <30 and <50 nmol/L, respectively, were higher than those presented in most reports of Caucasian women at a similar latitude including in the Netherlands (5), Canada (6), the United Kingdom (7), and Boston (8). In Scotland (57°N), 21.5% of concentrations were <25 nmol/L at 19 wk of gestation (39) compared with 11% of concentrations in the current cohort. Notwithstanding the ongoing debate about target cutoffs for 25(OH)D, international comparisons of vitamin D status have been hampered for too long by incomplete reporting. To facilitate comparison, we have described the prevalence below all currently debated 25(OH)D thresholds including 25, 30, 40, 50, and 75 nmol/L. On the basis of WHO criteria (40), the prevalence of vitamin D deficiency in the SCOPE Ireland pregnancy cohort could be categorized as a mild (17–19.9%) or severe (≥40%) public health problem depending on the 25(OH)D threshold (30 or 50 nmol/L) that is chosen to indicate increased risk of vitamin D deficiency, thereby illustrating the importance of transparent reporting.

Compared with nationally representative data (37), the current cohort had higher risk of vitamin D deficiency [25(OH)D concentration <30 nmol/L] than was shown for nonpregnant women aged 18–50 y (17% compared with 12%, respectively). Dietary intakes of vitamin D were not available, but data on vitamin D–containing supplement use were carefully recorded and specifically coded for the current analysis. The average vitamin D intake in women aged 18–45 y is ~3 µg/d (41). This amount would compute to an estimated average intake of ~8 µg/d in the subgroup of women in the current cohort who took 5 µg vitamin D₃/d as a supplement, of whom 94% maintained serum 25(OH)D concentrations >30 nmol/L through the entire year. This result is in close agreement with the current estimated average requirement of 10 µg/d for the prevention of 25(OH)D concentrations <30 nmol/L (13) and with our previous requirement estimate in young adults (42). A recent dose-response randomized controlled trial in Canada (43) showed that circulating 25(OH)D concentrations did not decline <30 nmol/L in pregnant and postpartum women who were taking 10 µg vitamin D₃/d. The achievement of 50 nmol/L required ≥25 µg vitamin D₃/d in the study and in another dose-response trial in pregnant women from New Zealand (44), which was also in agreement with our data and other data in nonpregnant adults (45).

Women in Ireland are not currently recommended by the authoritative agency to take vitamin D supplements during pregnancy. However, like other results (4, 5, 46, 47), our data emphasize that supplements are effective only in individuals who choose to take them. People who are the most vulnerable in the population (e.g., dark-skinned individuals who do not take a supplement) are at highest risk of deficiency. Although the subsample of dark-skinned women in this cohort was small, the very high prevalence of vitamin D deficiency in women of non-European origin who did not use supplements (70%) or who had 25(OH)D concentrations <30 nmol/L highlighted the significance of ethnicity as a risk factor during pregnancy. There is an urgent requirement for food-based strategies to increase the mean population intake to ≥10 µg/d and to prevent 25(OH)D concentrations falling to <30 nmol/L, which would benefit all of society, including women during pregnancy.

Our study observed a protective association, which was equivalent to a 36% reduction, of a composite outcome of pre-eclampsia plus SGA that was indicative of uteroplacental dysfunction in

### TABLE 3

<table>
<thead>
<tr>
<th>25(OH)D, nmol/L</th>
<th>All</th>
<th>Summer</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 1768)</td>
<td>(n = 734)</td>
<td>(n = 1034)</td>
</tr>
<tr>
<td>&lt;25</td>
<td>11</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>&lt;30</td>
<td>17</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>&lt;40</td>
<td>31</td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td>&lt;50</td>
<td>44</td>
<td>26</td>
<td>57</td>
</tr>
<tr>
<td>&lt;75</td>
<td>75</td>
<td>63</td>
<td>83</td>
</tr>
</tbody>
</table>

1Values are percent of n. Seasons were defined as follows: summer, June through October; winter, November through May. SCOPE, Screening for Pregnancy Endpoints; 25(OH)D, 25-hydroxyvitamin D.
TABLE 4
Effect modifiers of 25(OH)D in women in the SCOPE Ireland pregnancy cohort at 15 wk of gestation (n = 1768)1

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Mean ± SD</th>
<th>Adjusted mean difference (95% CI)</th>
<th>P</th>
<th>Winter P</th>
<th>Summer P</th>
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<tr>
<td>Winter</td>
<td>1034</td>
<td>49.6 ± 24.6</td>
<td>Reference</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Summer</td>
<td>734</td>
<td>66.7 ± 24.3</td>
<td>15.5 (13.4, 17.6)</td>
<td>&lt;0.0001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Vitamin D-supplement use</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>1054</td>
<td>69.7 ± 23.6</td>
<td>Reference</td>
<td>—</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Yes</td>
<td>714</td>
<td>47.8 ± 23.6</td>
<td>20.1 (18.5, 22.7)</td>
<td>&lt;0.0001</td>
<td>23.9 (21.0, 26.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>White</td>
<td>1727</td>
<td>57.2 ± 25.8</td>
<td>Reference</td>
<td>—</td>
<td>Reference</td>
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<tr>
<td>Other</td>
<td>41</td>
<td>36.1 ± 20.0</td>
<td>−19.3 (−25.4, −13.2)</td>
<td>&lt;0.001</td>
<td>−14.9 (−23.4, −6.5)</td>
<td>0.001</td>
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<td>Smoking status</td>
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<tr>
<td>No</td>
<td>1244</td>
<td>57.7 ± 25.9</td>
<td>Reference</td>
<td>—</td>
<td>Reference</td>
<td>Reference</td>
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<tr>
<td>Yes</td>
<td>177</td>
<td>47.1 ± 24.4</td>
<td>−7.0 (−10.5, −3.6)</td>
<td>&lt;0.0001</td>
<td>−7.1 (−11.4, −2.7)</td>
<td>0.002</td>
</tr>
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<td>BMI (continuous measure)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>215</td>
<td>49.4 ± 25.7</td>
<td>Reference</td>
<td>—</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>≥4 times/wk</td>
<td>322</td>
<td>62.1 ± 25.2</td>
<td>5.3 (1.1, 9.5)</td>
<td>0.013</td>
<td>6.1 (1.0, 11.2)</td>
<td>0.019</td>
</tr>
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<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1271</td>
<td>54.8 ± 25.5</td>
<td>Reference</td>
<td>—</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Yes</td>
<td>41</td>
<td>63.9 ± 23.3</td>
<td>−7.3 (−0.05, 14.6)</td>
<td>0.052</td>
<td>4.9 (−4.2, 14.1)</td>
<td>0.289</td>
</tr>
</tbody>
</table>

1Seasons were defined as follows: summer, June through October; winter, November through May. Multiple linear regression was used to explore predictors for concentrations of 25(OH)D at 15 wk of gestation. SCOPE, Screening for Pregnancy Endpoints; 25(OH)D, 25-hydroxyvitamin D.

women with 25(OH)D concentrations >75 nmol/L. Almost 1 in 20 pregnancies in the European Union are complicated by pre-eclampsia, which is the most prevalent cause of maternal mortality in Europe, accounting for 17–24% of all maternal deaths, and is associated globally with ~80,000 maternal and >500,000 infant deaths annually (48). In the longer term, pre-eclampsia is also associated with increased risk of cardiometabolic diseases later in a woman’s life (49), and because gestational hypertension, with or without pre-eclampsia, creates a predisposum to growth restriction and an SGA birth, the prevention of pre-eclampsia is of paramount importance for healthy prenatal and postnatal growth.

Several studies have reported associations between low vitamin D status and adverse perinatal outcomes (10–12). However, for several reasons, including a frequent reliance on retrospective data, inadequate clinical phenotyping, the variability in 25(OH)D analyses, and an inconsistency in the definition of vitamin D deficiency, the data have often been contradictory. In their updated Cochrane review of intervention studies in pregnancy of vitamin D (with or without calcium), De-Regil et al. (50) showed moderate evidence for a role of vitamin D supplementation in the prevention of pre-eclampsia, low birth weight, and preterm birth but urged caution in the clinical interpretation of the data because

TABLE 5
Association of total 25(OH)D at 15 wk of gestation with pre-eclampsia, SGA birth, and pre-eclampsia plus SGA as a composite indicator of uteroplacental dysfunction in nulliparous women in the SCOPE Ireland pregnancy cohort (n = 1768)1

<table>
<thead>
<tr>
<th></th>
<th>Pre-eclampsia (n = 68)</th>
<th>SGA (n = 190)</th>
<th>Composite pre-eclampsia plus SGA (n = 240)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted</td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Serum 25(OH)D, nmol/L</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>≤30</td>
<td>1.01 (0.53, 1.93)</td>
<td>0.74 (0.35, 1.54)</td>
<td>1.12 (0.73, 1.72)</td>
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<td>40</td>
<td>0.90 (0.52, 1.56)</td>
<td>0.68 (0.36, 1.26)</td>
<td>1.09 (0.75, 1.57)</td>
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<td>50</td>
<td>0.77 (0.46, 1.31)</td>
<td>0.59 (0.33, 1.06)</td>
<td>1.04 (0.74, 1.46)</td>
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<td>0.5–75</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>≥75</td>
<td>0.44 (0.21, 0.92)</td>
<td>0.49 (0.23, 1.05)</td>
<td>0.62 (0.40, 0.96)</td>
</tr>
</tbody>
</table>

1All values are ORs (95% CIs). Logistic regression was used to examine the association between total 25(OH)D and risk of pre-eclampsia and SGA birth and the composite outcome of pre-eclampsia and SGA as indicative of uteroplacental dysfunction. Models were adjusted for data collected at the 15-wk visit including supplementation, the season of sampling, the number of summer days during pregnancy, recreational walking, and job status. Additional adjustment for socioeconomic index, maternal age, BMI, smoking, alcohol use, education, ethnic origin, and marital status did not change the results materially. SCOPE, Screening for Pregnancy Endpoints; SGA, small for gestational age; 25(OH)D, 25-hydroxyvitamin D.
of low quality, the absence of the reporting of adverse effects, and high risk of bias in most studies. Two systematic reviews of 25(OH)D and pregnancy outcomes in observational studies (51, 52) highlighted the limitations imposed by high levels of heterogeneity and decided that the evidence was insufficient to draw firm conclusions. Clearly, many gaps remain in the investigation of vitamin D and perinatal outcomes.

Aghajafari et al. (51) were persuaded that, because of the mechanistic underpinning and biological plausibility of the associations between vitamin D and metabolic abnormalities, including hypertension, plus the relative consistency in the observational data and the likelihood that low 25(OH)D preceded the adverse outcome (thereby reducing the likelihood of reverse causation), intervention studies with defined outcomes were warranted. However the authors cautioned that these defined outcomes would only be valuable if comprehensive data were collected on nutrition, clinical characteristics, family histories, and relevant lifestyle factors such as sun exposure. Similarly, Thorne-Lyman and Fawzi (52) concluded that the evidence was insufficient to draw firm conclusions other than that intervention studies with defined outcomes in pregnancy were warranted in populations with a high prevalence of deficiency and at doses that were sufficient to achieve 25(OH)D targets.

At this point, we know that maternal vitamin D deficiency is completely preventable. The question of whether there is a detectable therapeutic window for an additional benefit if every woman who participates in an intervention study is prevented from becoming deficient [at a minimum of 30 nmol 25(OH)D/L] is a critical ethical and methodologic issue in terms of designing and implementing placebo-controlled vitamin D–intervention studies in pregnant women. Furthermore, there are many unanswered questions, and few investigators have systematically addressed the issue of habitual calcium intake in conjunction with low vitamin D status as evidence by elevated parathyroid hormone concomitant with low 25(OH)D (53).

Our study makes a significant contribution to the evidence base because of the quality of the study design and implementation, the clinical phenotyping, biobanking protocols, and accredited analytic data. However, we could not rule out the possibility of reverse causation. The detailed description of the clinical modifiers for 25(OH)D showed that, in addition to the season and ethnicity, lifestyle variables, such as current smoking, and recreational activity, such as regular walking, are potentially important. Because these clinical and lifestyle factors are also implicated in the risk profile that predisposes individuals to adverse perinatal outcomes (33), it is important that the factors are acknowledged as contributing both to low serum 25(OH)D concentrations and increased risk of disease. In our view, adjustment for these factors in the statistical analysis would have been unlikely to fully account for them in the pathologic process.

From an analytic point of view, these are the largest and most comprehensive data on 3-epi-25(OH)D3 in pregnancy to our knowledge. Variations in 3-epi-25(OH)D3 tracked 25(OH)D3 concentrations on a month-by-month basis. The question of whether 3-epi-25(OH)D3 elicits independent biological effects can only be further investigated when the purified compound can be manufactured at a standard and quantity that are suitable for human consumption in clinical studies. We also detected 25(OH)D3 in almost 100% of the cohort and showed an inverse association between 25(OH)D2 and 25(OH)D3 in the subgroup of women who consumed vitamin D2 supplements. With regard to the source of 25(OH)D2 in the diet of nonusers of supplements, we assume it arose from voluntary fortification and endogenous sources, such as mushrooms, similar to what was shown in a previous report in Irish adults (38).

In conclusion, we report a protective association of a 25(OH)D concentration >75 nmol/L and a reduced risk of uteroplacental dysfunction as indicated by a composite outcome of SGA and pre-eclampsia. Well-powered vitamin D intervention studies in pregnancy are warranted; however, there are significant ethical and design considerations.

The authors’ responsibilities were as follows—MEK and LCK: designed the research; JYZ and MK: conducted the sample analysis; MEK, JYZ, and ASK: conducted the data analysis; MEK and JYZ: wrote the manuscript; MEK: was responsible for the final content of the manuscript; and all authors: read and approved the final draft of the manuscript. None of the authors reported a conflict of interest related to the study.

REFERENCES


30. Holick MF, Biancuzzo RM, Chen TC, Klein EK, Young A, Bibuld D, Reitz R, Salameh W, Ameri A, Tannenbaum AD. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J Clin Endocrinol Metab 2008;93:1535–42.


40. Black LJ, Walton J, Flynn A, Cashman KD, Kiely M. Small increments in vitamin D intake by Irish adults over a decade show that strategic initiatives to fortify the food supply are needed. J Nutr 2015;145:969–76.


