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Vitamin D and Pre-Eclampsia: Original Data, Systematic Review and Meta-Analysis

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Key Words
Vitamin D · 25-Hydroxyvitamin D · Supplementation · Pre-eclampsia

Abstract
Background/Aims: Vitamin D may protect from pre-eclampsia through influences on immune modulation and vascular function. To evaluate the role of vitamin D in the development of pre-eclampsia, we conducted a systematic review and meta-analysis including novel data from 2 large-scale epidemiological studies. ***Methods:*** PubMed, EMBASE and the Cochrane Central Register of Controlled Trials were searched for prospective observational studies of association between vitamin D supplementation or status (measured by maternal 25-hydroxyvitamin D, 25(OH)D) with a subsequent risk of pre-eclampsia, or randomised controlled trials using vitamin D supplementation to prevent pre-eclampsia. The Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA) and the Avon Longitudinal Study of Parents and Children (ALSPAC) were included in meta-analyses with published studies. ***Results:*** Mothers receiving vitamin D supplementation earlier in pregnancy had lower odds of pre-eclampsia [pooled odds ratios (OR) 0.81 and 95% confidence interval (CI) 0.75–0.87, $p = 2.4 \times 10^{-8}$, 2 studies] in the meta-analysis of published studies with HCCSCA. The meta-analysis of published studies with ALSPAC suggested an association between higher serum 25(OH)D levels and a reduced risk of pre-eclampsia (pooled OR 0.52 and 95% CI 0.30–0.89, p = 0.02, 6 studies). Randomised trials of supplementation were suggestive of protective association (pooled OR 0.66 and 95% CI 0.52–0.83, p = 0.001, 4 studies). ***Conclusions:*** This study suggests that low maternal serum 25(OH)D concentrations increase pre-eclampsia risk and that vitamin D supplementation lowers this risk. The quality of evidence is insufficient to determine a causal association, which highlights the need for adequately powered clinical trials.

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Introduction

Pre-eclampsia is a life-threatening condition of pregnancy defined by the gestational onset of hypertension and proteinuria [1]. It affects 2–8% of first-time pregnant women, has marked international variation [2] and is responsible for up to 25% of maternal deaths worldwide [3]. Vitamin D is emerging as a promising agent for pre-eclampsia prevention [4]. Vitamin D deficiency is highly prevalent in women of reproductive age and in pregnant mothers [5]. If proven effective, the population level benefits of vitamin D supplementation would be substantial and likely to impact the long-term health of offspring [6].

Vitamin D is a seco-steroid pro-hormone which, for biological activation, undergoes two successive hydroxylations, firstly to 25-hydroxyvitamin D (25(OH)D), a nutritional biomarker for vitamin D status, and secondly to the active hormonal metabolite 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), i.e. calcitriol. Calcitriol exerts the hormonal action via binding to nuclear vitamin D receptors, which are present throughout the body, including pregnancy-specific tissues such as the placenta and uterine placental bed (decidua). The placenta and decidua as well as other important target cells such as immune and endothelial cells have the molecular machinery for local production of calcitriol [7, 8].

Pre-eclampsia is thought to originate in early pregnancy when the maternal immune system limits placental invasion in mothers vulnerable to cardiovascular disease. Calcitriol can be considered a pregnancy-supporting factor [9] that could work through several mechanisms to reduce pre-eclampsia risk, including a direct influence of calcitriol on implantation, placental invasion and angiogenesis [10, 11]. It is also believed to be important in directing immune responses by dendritic cells and macrophages at the fetal-placental interface as well as immunological adaptation by the mother to reduce the risk of infection and inflammation [4, 11].

Compared to normal pregnancies, vitamin D metabolism is markedly altered in pre-eclampsia. This may be due to reduced placental 1α-hydroxylase activity [12] resulting in lower circulating calcitriol concentrations compared to normotensive or chronically hypertensive pregnant women [13, 14]. Vitamin D status is reportedly lower in pre-eclamptic mothers at the time of diagnosis [13, 15], but also before disease onset in some studies [16, 17].

In this paper, we investigate the association between vitamin D supplementation and status with pre-eclampsia risk in 2 large population studies: 59,789 participants from the Hungarian Case-Control Surveillance System of Congenital Abnormalities (HCCSCA) and 5,058 participants from the Avon Longitudinal Study on Parents and Children (ALSPAC). We also undertook a systematic review and meta-analysis of all published prospective and intervention studies in order to evaluate the consistency and quality of evidence investigating an association between vitamin D supplementation and status in early pregnancy with pre-eclampsia risk.

Methods

Associations of vitamin D status during pregnancy with later pre-eclampsia were evaluated using novel data from 2 large-scale epidemiological studies. These data were incorporated into meta-analyses of previously published prospective and intervention studies that were identified following a systematic review.

The Hungarian Case-Control Surveillance System of Congenital Abnormalities

The HCCSCA was carried out from 1980 to 1996 and consisted of 22,843 cases with congenital abnormality (CA) and 38,151 sex-, birth-week- and district-of-residence-matched controls with no CA [18, 19]. Cases were identified through the Hungarian Congenital Abnormality Registry (coverage of approx. 90%) and controls from the National Birth Registry of the Central Statistical Office. For the purpose of these analyses, we excluded mothers undergoing elective termination of pregnancy (102 case-mothers) or multiple pregnancies (421 case- and 410 control-mothers). Due to the suspected influences of antiepileptic mediation on vitamin D metabolism, those with diagnoses of epilepsy were also excluded (98 case- and 92 control-mothers) as well as 33 (14 case- and 19 control-mothers) due to missing data on the starting point for supplementation or pre-eclampsia onset and 53 (20 case- and 33 control-mothers) with pre-eclampsia onset recorded on or before the first 3 months of pregnancy. After these exclusions, there were 22,189 and 37,600 mothers of CA cases and controls, respectively, available for our analysis.

Information on maternal characteristics (including age, parity and employment), pregnancy complications, medications and use of supplemental vitamins was obtained from 'prenatal maternity logbooks' filled in at visits to prenatal care clinics (21.0% of the case-mothers and 25.2% of the control-mothers), through retrospective structured questionnaires sent to the mother (55.8% of case-mothers and 32.5% of control-mothers) or from concordant information from both sources (23.1% of case-mothers and 42.4% of control-mothers) [20, 21]. The majority of mothers returned their logbooks and questionnaires, with home visits done by regional nurses for all non-respondent case mothers. Nurses visited only 200 non-respondent and 600 respondent control mothers in 2 validation studies [22], as further follow-up was considered by the ethical committee to be disturbing to the parents of all healthy children. Nearly 100% of the pregnant women visited antenatal-care clinics, as both maternity allowance and maternity leave were conditional to these visits. The first visit was typically between the 6th and 12th gestational week, with an average 7 visits per pregnancy. Overall, necessary information was available on 96.3% of cases (84.4% from a reply to mailing and 11.9% from a nurse visit)

and 83.0% of controls (82.6% from a reply, and 0.4% from a visit). Information on maternal characteristics was available for all mothers with data on supplement intake. The Central Ethical Committee of the Hungarian Ministry of Health approved the methodology of the HCCSCA, the visits to non-respondent case mothers at home and the recording of personal data. Informed consent was signed by 98% of the mothers and the details on the remaining subjects were deleted, leading to their exclusion.

The routine vitamin D supplementation recommendation to all pregnant women was 3,000 IU/week (corresponding to 430 IU/day) from the 20th gestational week. Several available multivitamin supplements also contained vitamin D (100–400 IU/day), and these were coded separately. All available single vitamin preparations contained cholecalciferol (vitamin D_3), while multivitamins contained either cholecalciferol or ergocalciferol (vitamin D_2). In our analyses, only mothers with a supplementation start time (recorded by gestational month) before the diagnosis of pre-eclampsia were considered. Maternal parity was based on the number of previous pregnancies, coded as 0, 1–2 or at least 3. Maternal employment was coded as professional, managerial, skilled worker, semi-skilled worker, unskilled worker, housewife or other (student/retired/unemployed) [18].

Blood pressure (BP) and proteinuria (dipstick screening test) were measured during visits to prenatal-care clinics. After the 20th gestational week, all mothers with proteinuria were referred for a detailed laboratory examination. Proteinuria was defined as >300 mg/24 h, with pre-eclampsia diagnosed if accompanied by hypertension (at least two measures >140/90 mm Hg).

The Avon Longitudinal Study on Parents and Children

The ALSPAC is a prospective population-based pregnancy cohort study that recruited 14,541 pregnancies resident in Avon, UK, with expected dates of delivery 1st April 1991 to 31st December 1992 (http://www.alspac.bris.ac.uk) [23]. There were 13,617 women with a live singleton birth who consented to have their obstetric data extracted from medical records. Ethical approval was obtained from the ALSPAC Law and Ethics Committee and the local Research Ethics Committee. For this study, we defined our eligible sample as women for whom we had sufficient information to determine the incidence of pre-eclampsia and for whom measures of 25(OH)D were available before diagnosis (n = 5,058).

Information to determine incidence of pre-eclampsia was extracted from antenatal records by research midwives. The median number (interquartile range) of BP measurements in pregnancy was 14 (11, 16) and that of urine measurements was 11 (10, 14). Diagnosis of pre-eclampsia was based on the criteria of the International Society for the Study of Hypertension in Pregnancy [24] as: BP ≥140/≥90 mm Hg, measured on at least two occasions after 20 weeks of gestation, with proteinuria (≥300 mg/24 h) occurring at the same time as the elevated BP. $25(OH)D_3$ and $25(OH)D_2$ concentrations in plasma or serum were measured with high-performance liquid chromatography tandem-mass spectrometry, with coefficients of variation for the assay <10% across a working range of 1–250 ng/ml [25]. Total 25(OH)D was calculated as the sum of $25(OH)D_3$ and $25(OH)D_2$.

Information on maternal age, ethnicity, education, physical activity and pre-pregnancy weight and height were obtained from questionnaire responses completed in early pregnancy. Ethnicity was categorized into white and non-white due to the small number of women from a non-white ethnic background. Education was categorised as: Certified Secondary Education [CSE, exams that used to be taken at age 16 by pupils considered less able than those taking ordinary O-level exams], vocational training, O-levels, A-levels or a university degree. Physical activity in pregnancy was assessed at 18 weeks gestation, expressed in average metabolic equivalents as previously described [26], and categorised into fifths. Pre-pregnancy BMI was calculated using a self-reported weight measurement. Self-reported maternal pre-pregnancy weight correlated highly with the first antenatal-clinic weight recorded in the medical records (r = 0.96).

Search Strategy and Selection Criteria for Other Studies Investigating Vitamin D Status and Supplementation in Relation to Pre-Eclampsia

An electronic search of academic, peer-reviewed journals was carried out using the PubMed central and EMBASE databases (up to 13 March 2013) to identify all studies on the association between vitamin D and pre-eclampsia published as full text papers in the English language. A search strategy was developed combining the results of the two main topics of interest, i.e. vitamin D and pre-eclampsia, and search terms were exploded using MeSH field tags (online suppl. fig. 2; see www.karger.com/doi/10.1159/000358338 for all online suppl. material). The Cochrane Central Register of Controlled Trials was also searched according to this strategy, and further records were identified by screening relevant texts for additional citations and searching the authors' personal archives and bibliographies.

Abstracts of all studies identified on-line by the search were evaluated independently by two review authors (A.C. and E.H.). Full texts of relevant abstracts were then assessed, with any discrepancies being resolved through discussion. Full texts were reviewed for eligibility if they met the following 3 main criteria: the study (1) observed pregnant women and recorded outcomes related to pre-eclampsia, (2) measured either serum 25(OH)D concentrations or recorded the use of vitamin D supplementation during pregnancy and (3) was either a clinical trial using oral vitamin D supplementation or a prospective observational study with information on 25(OH)D concentration or vitamin D supplementation obtained for a period preceding the diagnosis of pre-eclampsia. Studies measuring $1,25(OH)_2D$ concentrations were not included since this active metabolite has a short half-life and is unsuitable for use as a biomarker for vitamin D intake/status [27].

Data Extraction

For relevant articles, the following information was recorded in a pre-defined spreadsheet: study design and population, geographical location of study, entry criteria, definition of pre-eclampsia, 25(OH)D assay used (if relevant) or frequency and dosage of vitamin D supplementation, month of 25(OH)D measurement/supplementation and any adjustments included in the final models. For intervention studies, information about further relevant factors was recorded, such as randomisation methods and blinding. Studies were classified into 3 groups, those investigating the prospective association between (1) vitamin D status (measured by 25(OH)D) and incident pre-eclampsia, (2) vitamin D supplementation and incident pre-eclampsia and (3) interventions using vitamin D supplementation to prevent pre-eclampsia.

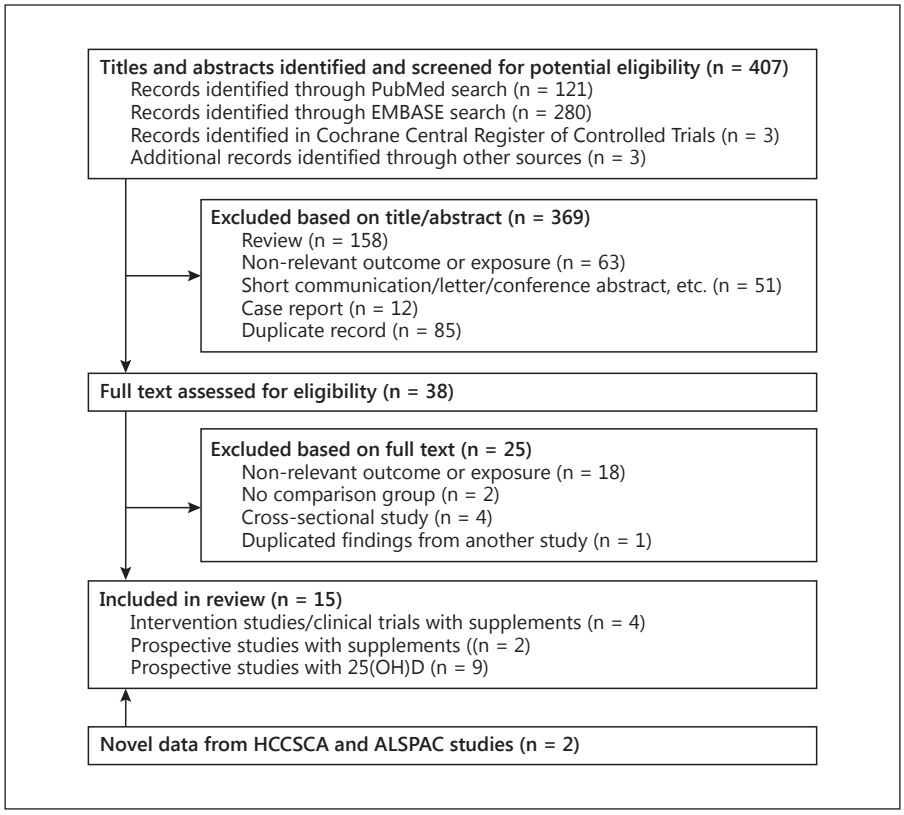

Fig. 1. Study selection process.

Statistical Analysis

In the HCCSCA, analyses were done separately for the CA case and control mothers. Vitamin D supplementation was considered only if taken before the diagnosis of pre-eclampsia, and was then analysed in dosage models comprising (1) 4 categories: no supplementation, multivitamin only, vitamin D supplementation only and vitamin D supplementation + multivitamin or higher dosage (>430 IU/day) in a single vitamin supplement and (2) 2 categories: no vitamin D supplementation versus any vitamin D supplementation. The main models used logistic regression adjusted for maternal age, parity and maternal employment. Information from other possible confounders such as BMI could not be included due to a lack of information. The likelihood ratio test or a trend test was used to calculate p values.

In the ALSPAC, vitamin D status was categorised as (1) <37.5 versus ≥37.5 nmol/l, (2) <50 versus ≥50 nmol/l and (3) ≥25 to <50, ≥50–75 and ≥75 nmol/l. Models were run unadjusted and also adjusted for maternal age, BMI, ethnicity, physical activity, parity, education, gestational age at time of blood sampling for 25(OH)D and season of sampling (by trigonometric sine-cosine regression with the date of sampling). For women with more than one 25(OH)D pregnancy measure, the one taken earliest in pregnancy was chosen. Only measures before 20 weeks of pregnancy were included in the analyses.

To increase efficiency and minimise selection bias, multivariate multiple imputation was used to impute missing values of covariables for eligible women, including all exposures, covariables, outcomes and potential predictors of missing data (birth weight and gestational age at birth) in the imputation equations [28]. Switching regression in STATA was used, as described by Royston [28]. Twenty cycles of regression switching were carried out and 30 imputation datasets were generated. Distributions of imputed variables in the imputed datasets and the observed data (with no imputation) were very similar and the results of the complete case analyses were not substantively different from those of the multivariate multiple imputation, but were less precisely estimated.

Data Synthesis

Where appropriate, data from individual studies were pooled using fixed-effect meta-analyses with the Mantel and Haenszel method. Statistical heterogeneity was assessed using the I^2 statistic. In the presence of heterogeneity, random-effects meta-analysis models were used with the method of DerSimonian and Laird.

When available from the original reports, odds ratios (OR) and 95% confidence intervals (CI) were used, and were otherwise calculated from other reported data. The direction of effect was reversed where appropriate, so that all OR related to the odds of pre-eclampsia in vitamin D-sufficient pregnant women compared to those who were considered insufficient (observational studies), or the odds of pre-eclampsia in the treatment group compared to the control group (intervention studies). To minimise bias, the main meta-analyses on observational studies of 25(OH)D and pre-eclampsia risk included only studies reporting confounder-adjusted OR. Meta-regression was used to explore possible variation in the association between 25(OH)D and pre-eclampsia by study size, trimester, assay type

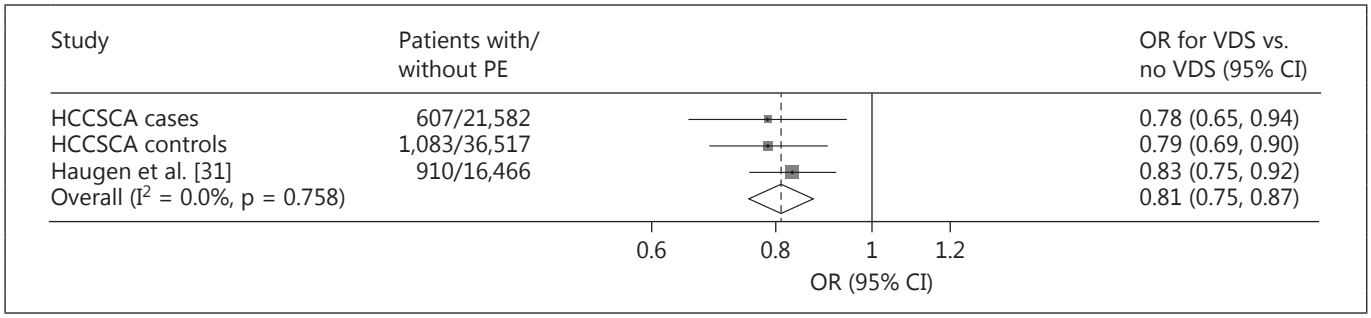

Fig. 2. Fixed effects meta-analysis on prospective observational studies of vitamin D supplementation (VDS) and subsequent risk of pre-eclampsia (PE).

Table 1. Vitamin D supplementation and risk of pre-eclampsia in the mothers from the HCCSCA (n = 59,789)

Vitamin D supplementation	Mothers to population controls (n = 37,600)				Mothers to CA cases (n = 22,189)			
	% (cases, n)	adjusted OR[c] (95% CI)	p value	p trend	% (cases, n)	adjusted OR[c] (95% CI)	p value	p trend
None	3.06 (790)	1 (reference)	–		2.93 (448)	1 (reference)	–	
Multivitamin[a]	2.89 (54)	0.92 (0.69, 1.22)	0.55		2.27 (22)	0.74 (0.48, 1.15)	0.18	
Vitamin D (3,000 IU/week)	2.39 (218)	0.76 (0.66, 0.89)	0.001		2.36 (129)	0.80 (0.66, 0.98)	0.03	
Vitamin D + multivitamins[b]	2.50 (21)	0.79 (0.51, 1.23)	0.3	0.0004	1.71 (8)	0.57 (0.28, 1.15)	0.12	0.007

[a] This supplied vitamin D ≤3,000 IU/week.
[b] Reported use of vitamin D 3,000 IU/week together with a multivitamin containing vitamin D or a reported higher dosage of vitamin D (≥6,000 IU/week).
[c] Adjusted for maternal age, parity and employment; unadjusted estimates were similar.

and latitude. Meta-analyses were also run separately for the two 25(OH)D thresholds (<37.5 and 50 nmol/l), including the relevant ALSPAC results in both analyses. Further sensitivity analyses excluded studies on high-risk populations or studies of severe pre-eclampsia.

Publication bias was evaluated through the inspection of funnel plots and the statistical testing of funnel-plot asymmetry [29]. All analyses were carried out using STATA (version 12).

Results

The initial search of the PubMed central, EMBASE and Cochrane databases and references within published papers identified 407 potentially relevant unique abstracts and titles for review. Of these, 369 were excluded based on their abstract only, most of them being either reviews, not relevant or else records duplicated between databases (fig. 1). Of 38 papers that underwent a full text review, 18 were excluded due to a lack of relevant data on outcome or exposure, 2 had no comparison group and 1 had duplicated findings from another study. Four studies were excluded because only information on the cross-sectional differences in 25(OH)D concentrations was presented. The definition of pre-eclampsia was consistent across all studies, with most of them stating a requirement that systolic BP was ≥140 mm Hg and/or diastolic BP was ≥90 mm Hg and proteinuria was at least 300 mg/24 h. In 2 of the studies [17, 30], the outcome considered was severe pre-eclampsia (online suppl. table 1).

Observational Studies on Vitamin D Supplementation and Risk of Pre-Eclampsia

In the HCCSCA, prevalence of pre-eclampsia was similar in mothers of CA cases and in controls (2.8 and 2.9%, respectively). In both groups, vitamin D supplementation earlier in pregnancy was associated with a reduced risk of developing pre-eclampsia, with some evidence for a dose-response effect (table 1).

We identified only 1 prospective observational study evaluating the association between vitamin D supple-

Table 2. Maternal serum 25(OH)D concentrations before pre-eclampsia onset in the ALSPAC (n = 5,058)

	Proportion in category, %	Incidence of pre-eclampsia, %	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
<25 nmol/l	4.7	2.7	1.90 (0.77, 4.64)	1.62 (0.65, 2.51)
≥25 to <50 nmol/l	30.2	2.3	1.60 (0.88, 2.88)	1.44 (0.79, 2.63)
≥50 to <75 nmol/l	30.9	2.1	1.44 (0.78, 4.64)	1.35 (0.73, 2.51)
≥75 nmol/l	34.2	1.4	Ref	Ref
Per 10 nmol/l		1.9	0.93 (0.86, 1.01) p trend = 0.06	0.95 (0.88, 1.02) p trend = 0.11

[a] Adjusted for maternal age, BMI, ethnicity, physical activity, parity, education, gestational age at the time of blood sampling for 25(OH)D and season of sampling.

mentation during pregnancy and pre-eclampsia risk [31]. The study compared mothers who had used vitamin D supplements before and/or during pregnancy with those who had not taken any vitamin D supplementation at any of these times, with an overall adjusted OR for pre-eclampsia of 0.83 (95% CI 0.75–0.92) for supplementation in the 1st trimester of pregnancy, with or without previous or later supplementation. When HCCSCA was combined in a meta-analysis with the study of Haugen et al. [31], the overall estimate suggested 19% lower odds (95% CI 25–13%, $p = 2.4 \times 10^{-8}$) of pre-eclampsia for mothers taking vitamin D supplements during pregnancy when averaged across dosages (fig. 2). The dosage of vitamin D in supplements taken by the mothers was <600 IU/day (most commonly, 200–430 IU/day) for the majority (>65%) of women, in both the HCCSCA and the study by Haugen et al. [31].

Observational Studies on 25(OH)D and Risk of Pre-Eclampsia

In ALSPAC, there were 99 incident cases of pre-eclampsia. Pre-eclampsia incidence was the highest for mothers with 25(OH)D <25 nmol/l and lowest for those with >75 nmol/l; however, evidence of a linear trend was weakened after adjustment for confounders (p = 0.11; table 2).

From 8 independent reports for prospective association between 25(OH)D and pre-eclampsia, 3 provided unadjusted estimates only and were consequently excluded from the meta-analyses (online suppl. table 1) [32–35]. In the 5 published confounder-adjusted studies included in the analyses [16, 17, 30, 35–37], 3 different 25(OH)D assays were used, and the threshold for low vitamin D status was defined as 37.5 nmol/l in 2 studies and 50 nmol/l in 3 studies. In 1 study, comparisons with the 'vitamin D sufficient' group (>75 nmol/l) were made. Two studies were of groups at a high risk of pre-eclampsia. All but 1 of the studies adjusted for season and BMI, with most of them also controlling for age, ethnicity, education or social class and gestational age for 25(OH)D assessment. 25(OH)D measures were done at the end of the 1st trimester in 1 study and other published studies included measures from the 2nd trimester.

When the ALSPAC was added to a random effects meta-analysis of studies investigating a relationship between 25(OH)D and pre-eclampsia, mothers with higher concentrations of 25(OH)D (as defined in each study) showed a decreased odds of developing pre-eclampsia compared with those who had lower 25(OH)D; pooled OR 0.52 (95% CI 0.30–0.89, p = 0.02). Study-specific OR for the association between 25(OH)D and pre-eclampsia ranged from 0.19 to 0.82 (fig. 3). There was evidence of heterogeneity between the studies (I^2 = 60%, 95% CI 4–84%, $p_{heterogeneity} = 0.03$), with the association between 25(OH)D and pre-eclampsia seen in studies carried out at latitudes south from 45° N (all from the USA) but not in studies located >45° N (OR 0.28 and 95% CI 0.12–0.65 and OR 0.80 and 95% CI 0.56–1.13, respectively, $p_{metaregression} = 0.04$; online suppl. fig. 3A). There were no systematic differences between studies supporting a stronger versus a weaker association in relation to study size, assay type or trimester ($p_{metaregression} > 0.16$ for all comparisons; online suppl. fig. 3B–D). Estimated effect was similar both for studies using 37.5 and 50 nmol/l as the cut-off for low concentrations (pooled OR 0.66 and 95% CI 0.32–1.35 and OR 0.60 and 95% CI 0.35–1.05, respectively) with precision reduced in both comparisons due to lower numbers (online suppl. fig. 4). Sensitivity analyses excluding one study of a high-risk population did not greatly affect the relationship between

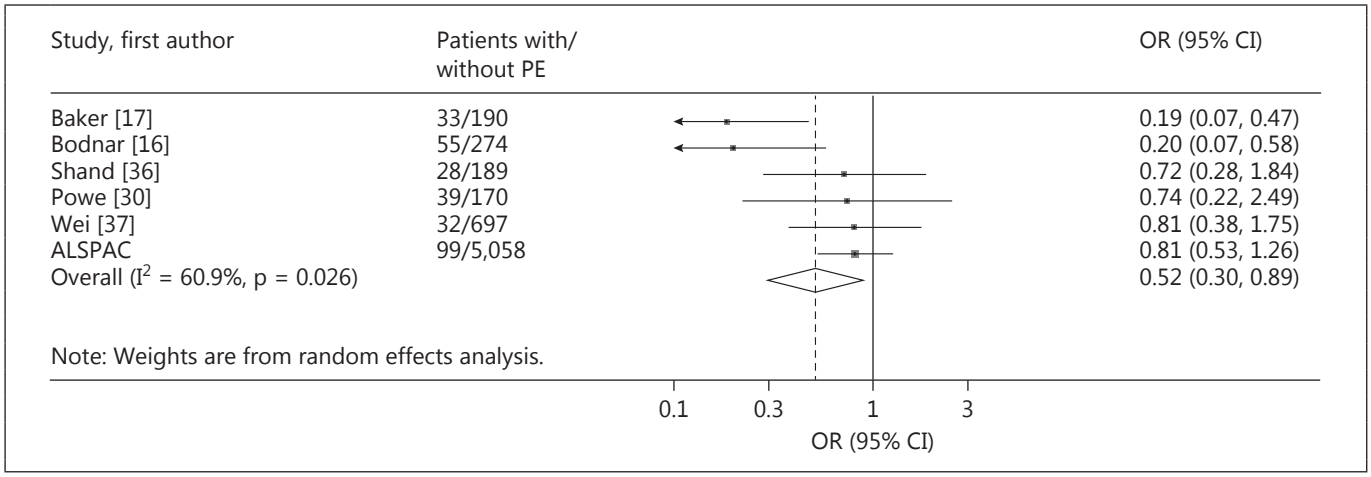

Fig. 3. Random effects meta-analysis on prospective observational studies of maternal serum 25(OH)D concentrations and subsequent risk of pre-eclampsia (PE).

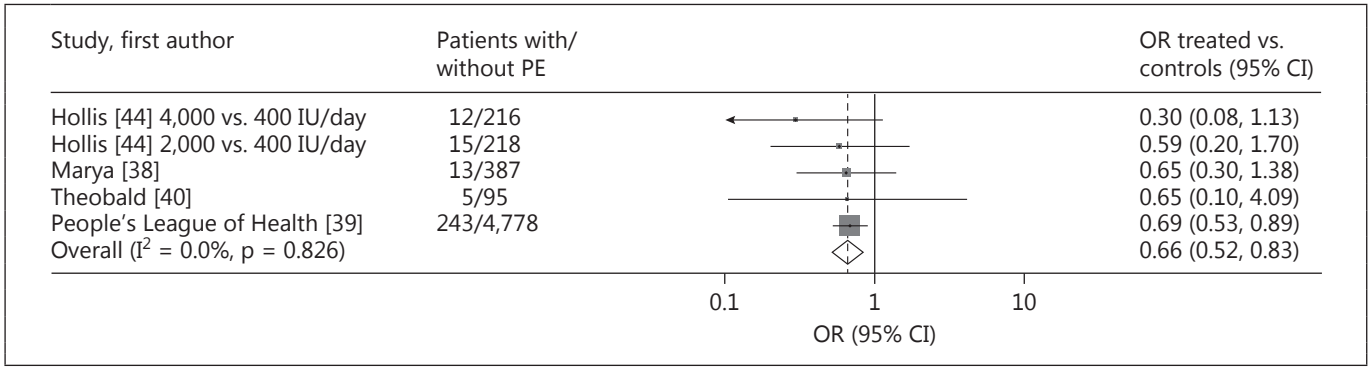

Fig. 4. Fixed effects meta-analysis on intervention studies using vitamin D supplementation to prevent pre-eclampsia (PE).

25(OH)D and pre-eclampsia (pooled OR 0.48 and 95% CI 0.25–0.92, p = 0.03), while the observed association was attenuated (pooled OR 0.63 and 95% CI 0.37–1.07, p = 0.08) when excluding 2 studies with the main outcome as severe pre-eclampsia. Effect estimates for all studies excluded from the meta-analyses due to lack of adjustment were suggestive of a protective association (OR 0.45–0.76). Examination of funnel plots did not provide any evidence for a publication bias (p = 0.23; online suppl. fig. 1).

Randomised Trials on Vitamin D Supplementation and Pre-Eclampsia

Of 4 trials included in the meta-analysis, 3 were placebo-controlled but unblinded studies [38–40]. The only blinded study [41] used supplementation with 400 IU/day as the comparison group, with the treatment groups receiving 2,000 and 4,000 IU/day. In the other 3 studies, the dosage of vitamin D supplementation in the treatment group ranged from 450 to 1,000 IU/day, and all used a supplement including other micronutrients (all with calcium, 1 with vitamin A and 1 with a multivitamin).

Combining the data available from these 4 trials showed a reduced odds of pre-eclampsia in women taking vitamin D supplementation compared with the control group, with a pooled OR of 0.66 (95% CI 0.52–0.83, p = 0.001). Individual study OR ranged from 0.44 to 0.69, with no statistical heterogeneity noted between the trials (I^2 = 0, 95% CI 0–48%, $p_{heterogeneity}$ = 0.83; fig. 4).

Discussion

The association between maternal vitamin D supplementation and status with the risk of pre-eclampsia was consistent across different types of studies, with evidence for benefits in prospective observational studies as well as in clinical trials. Two recent meta-analyses of observational studies also support an association between maternal serum 25(OH)D levels and pre-eclampsia, although in contrast to current study, neither of them restricted their analyses to confounder-adjusted studies and one of them included studies that measured concentrations at the time of pre-eclampsia [42, 43]. Several studies provided evidence for a dose-dependent association, with the recent trial by Hollis and Wagner [44] providing the most notable example. However, the causality of the association remains unproven, as the bulk of evidence was obtained from observational studies and only 1 of the intervention studies was blinded. Pre-eclampsia is potentially a life-threatening condition for both mother and fetus. Delivery of the placenta remains the only cure, but is often associated with severe prematurity for the neonate. Vitamin D is a promising candidate for pre-eclampsia prevention, and there is an urgent need for well-controlled randomised trials to test its effectiveness and safety.

Methodological Considerations

In this meta-analysis, potential for publication bias was reduced by inclusion of unpublished as well as published data, with the decision to include 2 large unpublished studies made a priori to carrying out analyses. Assays and cut-off points or contrasts used to examine vitamin D 'deficiency' varied between studies included in the meta-analysis, making quantification of the magnitude of association difficult. In the HCCSCA, it can be argued that factors affecting pre-eclampsia risk differ between pregnancies leading to offspring with CA compared to those with healthy outcomes; however, the observed association between vitamin D supplementation and pre-eclampsia was similar in both sub-sets. Despite restricting our analysis to studies where measurements of vitamin D were obtained before the diagnosis of pre-eclampsia, we cannot discount the possible influence of reverse causation [i.e. the influence of underlying pre-eclampsia on 25(OH)D concentrations]. Measurement of vitamin D exposure was done in most studies during the 2nd trimester, while only 1 study provided measures from the 1st trimester, and preconception measures were not available. Restriction of the sample to mothers with information on vitamin D supplementation reported before pre-eclampsia (compared to any stage of pregnancy) in the HCCSCA strengthened the association, which suggests that a false-positive association is unlikely. However, for studies assessing influences on pre-eclampsia risk by vitamin D status, where concentrations could be affected by less time spent outdoors due to the disease process, this would have been more likely to result in a type I error. With observational studies, it is not possible to exclude the possibility of residual confounding. While in the ALSPAC study we aimed for the inclusion of key confounding factors including physical activity (as a proxy for time spent outdoors), adjustments in previous publications were typically less comprehensive. Results from clinical trials remain inconclusive as most were of low quality, were not blinded and included other nutrients in addition to vitamin D. While secondary analyses evaluating the association between vitamin D supplementation (as a single ingredient preparation) and pre-eclampsia risk in the recent double-blind, randomised trial were suggestive of a protective association [44], the numbers of cases were small and the outcome included mothers with gestational hypertension as well as pre-eclampsia.

Mechanisms

There are several mechanisms by which vitamin D could potentially prevent or at least delay the progression to pre-eclampsia. For example, progression to pre-eclampsia may result from a multitude of immune and vascular defects. One potential mechanism relates to a defective control of effector T cells by regulatory T cells. This can lead to poor placental invasion, which in turn leads to the release of placental-derived vasoconstrictor factors and consequent maternal hypertension and proteinuria [45]. Calcitriol is believed to be important in maintaining and restoring immune homeostasis and tolerance. Vitamin D receptors on immune cells express key enzymes involved in the hormonal activation (CYP27B1) and catabolism (CYP24A1) of vitamin D metabolites, suggesting that the availability and effectiveness of calcitriol can be directly regulated by the cells of the immune system [46]. The net result of calcitriol on adaptive immune responses leads to a skewing towards a more tolerogenic status, which is a maternal immune adaptation required for the maintenance of a healthy pregnancy [47]. In vitro studies have demonstrated that calcitriol administration leads to an up-regulation of regulatory T cell responses while pro-inflammatory responses are typically down-regulated [46], constituting an adaptation to maternal tolerance that would reduce the risk of pre-eclampsia.

Vitamin D receptors on the heart and blood vessels suggest vitamin D has a cardio-protective effect, and calcitriol can influence endothelial and vascular smooth-muscle cell function as well as controlling inflammation and affecting the regulation of blood pressure through influences on the renin-angiotensin-aldosterone system [48]. Calcitriol may reduce pre-eclampsia risk through influences on angiogenesis [49]. This claim is supported by the known stimulatory effects of calcitriol on the expression of vascular endothelial growth factor in vascular smooth-muscle cells through vitamin D response elements in the vascular endothelial growth factor promoter [50]. Direct effects on the arterial wall by calcitriol may be important by preventing cholesterol uptake by macrophages and vascular smooth muscle proliferation; an athermanous pathology that is acutely observed in uteroplacental vessels in women with pre-eclampsia [51]. Therefore, vitamin D is likely to play an important role in the immune and cardiovascular changes necessary for a healthy pregnancy, highlighting the need to ensure that the existing public-health recommendations for vitamin D intake by pregnant mothers are endorsed.

Conclusions

This study has shown a consistent association between vitamin D and pre-eclampsia across different study types, supporting a role for vitamin D as a preventative agent against pre-eclampsia. However, due to the design and power of many of the trials included in our meta-analyses, this association is not conclusive and remains an important hypothesis that needs to be tested in a well-designed, randomised, controlled trial. If proven effective, vitamin D could provide cheap and safe prevention of pre-eclampsia. However, vitamin D deficiency is a correctable state that can affect the health of both mother and offspring also in other ways. While waiting for conclusive evidence that may support the proposed role for vitamin D in pre-eclampsia prevention, it is important to ensure that existing recommendations for vitamin D intake are systematically endorsed [52, 53].

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