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Exploring the concept of functional vitamin D deficiency in pregnancy: impact of the interaction between 25-hydroxyvitamin D and parathyroid hormone on perinatal outcomes

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Short running head: Vitamin D, parathyroid hormone and pregnancy

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Number of Figures: 1

Trial registration: The SCOPE pregnancy cohort is registered at the Australian, New Zealand Clinical Trials Registry (<http://www.anzctr.org.au>), ID: ACTRN12607000551493.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BP, blood pressure; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MAP, mean arterial pressure; PTH, parathyroid hormone; SCOPE, Screening for Pregnancy Endpoints study; SGA, small-for-gestational-age

1 **Abstract (298 words)**

2 **Background:** Associations of vitamin D with perinatal outcomes are inconsistent and few
3 have considered the wider calcium metabolic system.

4 **Objective:** To explore functional vitamin D deficiency in pregnancy by investigating
5 associations between vitamin D status, parathyroid hormone and perinatal outcomes.

6 **Design:** SCOPE (Screening for Pregnancy Endpoints) Ireland is a prospective cohort study of
7 low risk, nulliparous pregnant women. We measured serum 25-hydroxyvitamin D [25(OH)D]
8 and parathyroid hormone [PTH] at 15 weeks' gestation in 1754 participants.

9 **Results:** Mean \pm SD 25(OH)D was 56.6 ± 25.8 nmol/L (22.7 ± 10.3 ng/mL) and geometric
10 mean (95% CI) PTH was 7.84 (7.7, 8.0) pg/mL [0.86 (0.85, 0.88) pmol/L]. PTH was elevated
11 in 34.3% of women who had 25(OH)D <30 nmol/L and in 13.9% of those with 25(OH)D ≥ 75
12 nmol/L. While 17% had 25(OH)D <30 nmol/L, 5.5% had functional vitamin D deficiency,
13 defined as 25(OH)D <30 nmol/L plus elevated PTH. Elevated mean arterial pressure (MAP),
14 gestational hypertension, preeclampsia and small-for-gestational-age (SGA) birth were
15 confirmed in 9.2%, 11.9%, 3.8% and 10.6% of participants, respectively. In fully adjusted
16 regression models, neither low 25(OH)D nor elevated PTH alone increased risk of any
17 individual outcome. The prevalence of elevated MAP (19.1% vs. 9.7%) and SGA (16.0% vs.
18 6.7%) were highest ($P < 0.05$) in those with functional vitamin D deficiency compared to the
19 reference [25(OH)D ≥ 75 nmol/L and normal PTH]. The adjusted prevalence ratio (PR) and
20 relative risk (RR) (95% CIs) for elevated MAP and SGA were 1.83 (1.02, 3.27) and 1.53
21 (0.80, 2.93), respectively. There was no effect of functional vitamin D deficiency on the risk
22 of gestational hypertension [adjusted RR (95% CI); 1.00 (0.60, 1.67)] or preeclampsia
23 [adjusted RR (95% CI); 1.17 (0.32, 4.20)].

24 **Conclusions:** The concept of functional vitamin D deficiency, reflecting calcium metabolic
25 stress, should be considered in studies of vitamin D in pregnancy.

26 **Key words:** Vitamin D, 25-hydroxyvitamin D, parathyroid hormone, pregnancy, perinatal,
27 mean arterial pressure, gestational hypertension, preeclampsia, small-for-gestational-age

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28 **Introduction**

29
30 Pregnancy represents a period of particular nutritional vulnerability for the mother and
31 developing fetus and nutritional deficits can adversely affect perinatal outcomes. Low
32 vitamin D status [assessed by blood levels of 25-hydroxyvitamin D (25(OH)D)] in pregnancy
33 is prevalent worldwide, in Caucasian and other ethnicities (1-3). Although low 25(OH)D has
34 been associated with adverse perinatal outcomes including preeclampsia and small-for
35 gestational-age (SGA) birth (4, 5), there is inconsistency in the literature (6). Vitamin D
36 classically functions as part of the calcium metabolic system which maintains serum calcium
37 within a critical narrow physiological window (7). Despite this long recognised metabolic
38 connection, vitamin D and calcium are most often considered in isolation in terms of
39 perinatal outcomes, with a lack of consideration for interactive effects (8). Although
40 attenuated, the inverse relationship between 25(OH)D and calciotropic parathyroid hormone
41 (PTH) is maintained in pregnancy in spite of the many changes in vitamin D and calcium
42 metabolism (9). Secondary hyperparathyroidism refers to elevation of PTH resulting from
43 low 25(OH)D and/or low calcium intake (10) and represents functional vitamin D deficiency.
44 Scholl and colleagues described the concept of calcium metabolic stress in pregnancy, in
45 which adverse effects of vitamin D deficiency are mediated through a functional impact on
46 the calcium metabolic system (assessed by measurement of PTH) (11, 12). To the best of our
47 knowledge, these associations have not been replicated in a large, well-characterized
48 pregnancy cohort. We aimed to test the concept of calcium metabolic stress by exploring
49 associations of 25(OH)D, PTH and functional vitamin D deficiency at 15 weeks' gestation
50 with adverse perinatal outcomes, including elevated mean arterial pressure (MAP),
51 gestational hypertension, preeclampsia and SGA birth in a large cohort of low risk pregnant
52 women.

53 **Subjects and Methods**

54 **Study design and participants**

55 The Screening for Pregnancy Endpoints (SCOPE) study (www.scopestudy.net), an
56 international, multi-center, prospective cohort study was designed with the primary aim of
57 developing screening tests to predict adverse pregnancy outcomes, with preeclampsia as the
58 primary outcome variable (13). SCOPE Ireland recruited 1792 participants who were
59 attending antenatal care at Cork University Maternity Hospital, Cork, Ireland (51.9°N)
60 between March 2008 and February 2011. Full clinical and methodological study details have
61 been published previously (13). In summary, nulliparous women were eligible for inclusion
62 provided the pregnancy was a low risk singleton pregnancy at less than 16 weeks' gestation.
63 Exclusion criteria included pregnancies at increased risk of preeclampsia, SGA or
64 spontaneous preterm birth due to specific underlying medical conditions or medical history,
65 known major fetal anomaly or abnormal karyotype and interventions that could modify the
66 outcome of pregnancy, such as aspirin treatment.

67 Extensive data on family situation, lifestyle and demographics, including current smoking,
68 alcohol and drug use, supplement use, activity, employment and medical history were
69 collected by trained research midwives. As a pre-pregnancy measurement was not possible,
70 and given the well-established systematic bias in self-report of weight and height, maternal
71 height and weight at 15 weeks' gestation were measured for calculation of BMI (14). Two
72 consecutive blood pressure (BP) measurements were taken using a mercury or aneroid
73 sphygmomanometer and proteinuria was assessed by semi-quantitative automated dip-stick
74 reading. All demographic, anthropometric and clinical data were entered into a secure
75 internet deployed database (MedSciNet AB, Stockholm, Sweden). Non-fasting blood samples
76 were collected, processed to serum and stored at -80°C within 4 hours of collection.

77 SCOPE was conducted in accordance with the Declaration of Helsinki guidelines and ethical
78 approval was obtained from the Clinical Research Ethics Committee of the Cork Teaching
79 Hospitals [ECM5(10)05/02/2008]. SCOPE is registered at the Australian, New Zealand
80 Clinical Trials Registry (<http://www.anzctr.org.au>), ID: ACTRN12607000551493. Written
81 informed consent was provided by all participants at commencement of the study, which was
82 on average, at 15 weeks of gestation.

83 **Clinical definition of outcomes**

84 Perinatal outcomes were precise and predefined (13), with participants followed
85 prospectively throughout pregnancy and delivery. MAP was calculated from systolic and
86 diastolic BP measurements as $\text{diastolic BP} + [(\text{systolic BP} - \text{diastolic BP})/3]$, with $\text{MAP} > 90$
87 mmHg denoting elevated MAP. Gestational hypertension was defined as a systolic BP ≥ 140
88 mmHg and/or diastolic BP ≥ 90 mmHg on at least two occasions four hours apart after 20
89 weeks' of gestation but before the onset of labor. Preeclampsia was gestational hypertension
90 with either proteinuria (24 hour urinary protein ≥ 300 mg or spot urine protein:creatinine ratio
91 ≥ 30 mg/mmol creatinine or urine dipstick protein $\geq 2+$) or any multi-system complication of
92 preeclampsia. SGA birth was defined as a birth weight $< 10^{\text{th}}$ customized centile adjusted for
93 maternal height, booking weight and ethnicity as well as gestation at delivery and infant sex
94 (15).

95 **Biochemical Analysis**

96 *Serum 25(OH)D*

97 Measurement of 25(OH)D in our laboratory has been detailed previously (16). Briefly, total
98 25(OH)D was calculated by summation of individually quantified 25(OH)D₂ and 25(OH)D₃.
99 Serum 25(OH)D₂ and 25(OH)D₃ concentrations were measured in using liquid
100 chromatography-tandem mass spectrometry (LC-MS/MS) on a Waters Acquity UPLC

101 system coupled to an Acquity Triple Quadrupole (TQD)[®] mass spectrometer detector
102 (Waters, Milford, USA). Four levels of serum-based NIST (National Institute of Standards
103 and Technology) certified quality assurance material (SRM 972) were used for method
104 validation while quality control materials assayed in parallel to all samples were purchased
105 from Chromsystems (Munich, Germany). NIST calibrators (SRM 2972) were used
106 throughout the analysis. The limit of detection (LoD) for 25(OH)D₃ and 25(OH)D₂ was 0.31
107 and 0.44 nmol/L, respectively. The limit of quantitation (LoQ) for 25(OH)D₃ and 25(OH)D₂
108 was 1.03 and 1.43 nmol/L, respectively. Intra- and inter- assay CVs for both metabolites were
109 < 6% and < 5% respectively. The laboratory of the Cork Centre for Vitamin D and Nutrition
110 Research is accredited by the CDC Vitamin D Standardization Certification program and
111 participates in the Vitamin D External Quality Assessment Scheme (DEQAS) (Charring
112 Cross Hospital, London, UK).

113 *Serum PTH*

114 Serum intact PTH was measured using an ELISA (MD Biosciences Inc., Minnesota, USA) on
115 the automated Dynex DS2[®] ELISA processing platform (Dynex Technologies, Virginia,
116 USA). This two-site assay is designed to measure biologically intact PTH 1-84 and utilizes
117 two purified goat polyclonal antibodies, each specific to a distinct region on the PTH
118 molecule. A biotinylated antibody binds to mid-region and C-terminal PTH 39-84. The
119 detection antibody, a horseradish peroxidase conjugated antibody, binds N-terminal PTH 1-
120 34. PTH was measured in duplicate in 1497 participants and in singular in a further 257
121 participants as serum volume was insufficient for duplicate measurement. Geometric mean
122 PTH concentration did not differ between duplicate and single measurements (independent
123 samples t-test, $P = 0.10$) and single and duplicate measurements were collated for analysis,
124 giving a total of 1754 PTH measurements. Intra- and inter- assay CVs for intact PTH were <
125 5% and < 7% respectively.

126 **Statistical analysis**

127 Statistical analysis was performed using IBM SPSS® version 22.0 (IBM Corp., Armonk, NY,
128 USA) and SAS® version 9.4 (SAS Institute Inc., Cary, NC, USA) software for Windows™. A
129 full dataset, with no missing information, was available for all participants ($n = 1754$) and
130 analysis was carried out without imputation. PTH was natural log-transformed to an
131 approximately normal distribution and log PTH was used in analysis. Descriptive statistics
132 were prepared for participants and independent samples t-test or ANOVA were used as
133 appropriate to assess differences in mean concentrations of 25(OH)D and PTH within
134 maternal characteristics. Post-hoc comparisons used Bonferroni correction. 25(OH)D is
135 reported as mean \pm SD and PTH as geometric mean (95% CI). The 25(OH)D/PTH
136 relationship was graphically depicted by a scatter plot with Lowess curve (locally weighted
137 regression smoothing scatterplot). ANOVA with Bonferroni corrected post-hoc analysis was
138 performed to assess PTH concentrations across categories of 25(OH)D. Occurrence of
139 adverse outcomes was compared between 25(OH)D/PTH categories using Chi-square tests
140 (or Fisher's test as appropriate).

141 Associations between predictors and outcomes were assessed by log-Poisson regression, with
142 regression models constructed to examine the effects of low 25(OH)D and elevated PTH,
143 both individually and in combination, on adverse outcomes. While the full distribution of
144 25(OH)D was explored, and 25(OH)D and PTH were described across the range of 25(OH)D
145 values, we considered low vitamin D status as 25(OH)D < 30 nmol/L (7) and replete status \geq
146 75 nmol/L (17). These categories were used in regression models to allow simple comparison
147 between groups of interest i.e. those with a low and replete vitamin D status. In the absence
148 of pregnancy-specific reference ranges, we defined elevated PTH as greater than the 80th
149 percentile. However, to minimize potential influence of undiagnosed pathological conditions
150 relating to PTH, we excluded the top 2.5% of PTH concentrations. Thus, participants with

151 PTH between the 80th and 97.5th percentiles were classed as having elevated PTH. Reference
152 groups were 25(OH)D \geq 75 nmol/L and normal PTH (i.e. PTH \leq 80th percentile). Non-white
153 participants ($n = 40$) were excluded from regression models.

154 Separate log-Poisson regression models (12 in total) were built for each predictor-outcome
155 association based on both statistical goodness of fit of the model and clinical and theoretical
156 knowledge. To circumvent the problem of large standard errors obtained with standard
157 Poisson regression, we used Zou's modified Poisson approach which leads to the estimation
158 of robust error variance and produces correct confidence intervals (18). To obtain best fit
159 models the following steps were taken. Univariable analysis was used to assess the
160 association between each potential covariate and the outcome of interest, with inclusion
161 based on significance at $\alpha = 0.25$. A multivariable model including all significant
162 covariates from the univariate analysis was constructed. The association of interaction terms
163 with each outcome was examined, and interaction terms were included in multivariable
164 models if significant (at $\alpha = 0.10$) and clinically meaningful. To support the statistical
165 model building process clinical and theoretical knowledge of established associations
166 between variables along with directed acyclic graphs (DAGs) were used to develop a
167 theoretical framework of covariate-predictor-outcome relationships and to determine the role
168 of each covariate (e.g. confounder, intermediate, collider) in the model, aiding in decision
169 making during model development. This process resulted in an individual best-fit log-Poisson
170 regression model for each of the 12 predictor-outcome models. Although all associations
171 were examined using the same modified log-Poisson approach detailed above, because
172 25(OH)D, PTH and MAP were measured at the same time point associations are presented as
173 prevalence ratios (PR) and 95% CIs while associations of 25(OH)D and PTH with gestational
174 hypertension, preeclampsia and SGA are expressed as relative risks (RR) and 95% CIs.

175 **Results**

176

177 **Descriptive results**

178 Participant characteristics and 25(OH)D and PTH concentrations in the SCOPE Ireland
179 pregnancy cohort ($n = 1754$) are shown in **Table 1**. Mean \pm SD 25(OH)D concentration was
180 56.62 ± 25.8 nmol/L (22.7 ± 10.3 ng/mL) and geometric mean (95% CI) PTH was 7.84 (7.7,
181 8.0) pg/mL [0.86 (0.85, 0.88) pmol/L]. This predominantly white (97.7%) cohort had a mean
182 \pm SD age of 30.5 ± 4.5 years and BMI of 24.9 ± 4.2 kg/m². At 15 weeks of gestation, 10.0%
183 and 16.4% of participants, respectively, reported current smoking and alcohol consumption.
184 Multi-nutrient supplements were consumed by 40% of participants and this was associated
185 with significantly higher 25(OH)D and lower PTH concentrations in consumers ($P < 0.001$
186 for both). White ethnicity was also associated with higher 25(OH)D and lower PTH than
187 other ethnicities ($P \leq 0.001$ for both). Although season of entry was significantly associated
188 with 25(OH)D concentration ($P < 0.001$), PTH did not change with season. PTH increased
189 and 25(OH)D decreased with increasing BMI category at 15 weeks of gestation; however
190 post-hoc analysis revealed a significant decrease in 25(OH)D only in obese participants (BMI
191 ≥ 30 kg/m²) compared to those with a normal BMI. PTH concentration was highest in the
192 small proportion of participants (1.2%) with a BMI < 18.5 kg/m². Mean arterial pressure was
193 79.1 ± 7.6 mmHg and 9.2% of participants had elevated MAP (> 90 mmHg). The prevalence
194 of gestational hypertension, preeclampsia and SGA in the cohort were 11.9%, 3.8% and
195 10.6%, respectively.

196 **Associations of 25(OH)D, PTH and functional vitamin D deficiency with perinatal**

197 **outcomes**

198 The lowess curve in **Figure 1** illustrates a decrease in PTH across a broad range of increasing
199 25(OH)D concentrations, with a decline in rate of decrease evident between 40 - 50 nmol/L.
200 **Table 2** depicts PTH concentrations across the distribution of 25(OH)D, showing that PTH

201 decreased significantly with increasing 25(OH)D ($P < 0.001$). Elevated PTH occurred more
202 commonly ($P < 0.05$) in participants with 25(OH)D < 30 nmol/L (34.3%) than those ≥ 75
203 nmol/L (13.9%). In terms of vitamin D status, 44% of women had 25(OH)D < 50 nmol/L and
204 17% were below 30 nmol/L, while 25% were had 25(OH)D ≥ 75 nmol/L. In the stratified
205 analysis of 25(OH)D and PTH, the prevalence of functional vitamin D deficiency, defined as
206 25(OH)D < 30 nmol/l plus PTH $> 80^{\text{th}}$ percentile, was much lower, at 5.5%, with 11.4%
207 having a 25(OH)D < 50 nmol/L plus PTH $> 80^{\text{th}}$ percentile.

208 Associations of 25(OH)D, PTH and 25(OH)D/PTH groupings with elevated MAP are shown
209 in **Table 3**. The prevalence of elevated MAP was not significantly higher (12.0% versus
210 9.3%) in participants with 25(OH)D < 30 nmol/L compared with 25(OH)D ≥ 75 nmol/L ($P >$
211 0.05). While there was an association of elevated PTH with elevated MAP [PR (95% CI);
212 1.49 (1.08, 2.04)], this trend was attenuated with covariate adjustment. Stratification of
213 participants by 25(OH)D/PTH, shown in Table 3, revealed a prevalence of elevated MAP of
214 19.1% in those with functional vitamin D deficiency compared with 9.7% in the reference
215 group [25(OH)D ≥ 75 nmol/L and normal PTH] ($P < 0.05$), translating to an adjusted PR
216 (95% CI) of 1.83 (1.02, 3.27). Prevalence of elevated MAP did not increase with 25(OH)D $<$
217 30 nmol/L if PTH was not elevated [adjusted PR (95% CI); 0.91 (0.50, 1.65)].

218 **Table 4** shows the associations of 25(OH)D, PTH and 25(OH)D/PTH groupings with
219 gestational hypertension, preeclampsia and SGA birth. Having a 25(OH)D < 30 nmol/L
220 increased the risk of SGA in crude but not adjusted analysis. There was no association of
221 elevated PTH with gestational hypertension, preeclampsia or SGA. In combined
222 25(OH)D/PTH groupings, the prevalence of gestational hypertension was highest with
223 elevated PTH and did not vary with 25(OH)D (both 18%, $P > 0.05$). Having functional
224 vitamin D deficiency did not increase risk of gestational hypertension compared to the

225 reference, which was 25(OH)D \geq 75 nmol/L with normal PTH [adjusted RR (95% CI); 1.00
226 (0.60, 1.67)].

227 The lowest prevalence of preeclampsia (1.9%) was in those with 25(OH)D \geq 75 nmol/L and
228 normal PTH, compared to 5.0% when 25(OH)D \geq 75 nmol/L with elevated PTH ($P > 0.05$).

229 In those with 25(OH)D $<$ 30 nmol/L, the prevalence of preeclampsia did not differ depending
230 on PTH status ($P > 0.05$). Occurrence of preeclampsia was not significantly increased (4.3%
231 versus 1.9%) with functional vitamin D deficiency compared to the reference group ($P >$
232 0.05) [adjusted RR (95% CI); 1.17 (0.32, 4.20)].

233 The proportion of participants with a SGA newborn in the reference group (6.7%) was lower
234 than any other group, while 16% of those with functional vitamin D deficiency had a SGA
235 birth ($P < 0.05$). A 2-fold increase in risk [RR (95% CI); 2.38 (1.31, 4.33)] was attenuated
236 [adjusted RR (95% CI); 1.53 (0.80, 2.93)] in a fully adjusted model including BMI, smoking,
237 university education, job status, recreational walking and multi-nutrient supplementation. A
238 25(OH)D concentration \geq 30 - $<$ 75 nmol/L with elevated PTH was not associated with SGA
239 or any other outcome (data not shown). Regression models were repeated for all outcomes
240 using a 25(OH)D cut-off of $<$ 50 nmol/L; no associations were observed in multivariate
241 models (data not shown).

242

243 **Discussion**

244 Inconsistencies in associations of maternal vitamin D status and perinatal outcomes are
245 multifactorial (4, 5, 19) but it is likely that calcium-vitamin D interactions, largely ignored,
246 could be a critical consideration (8, 11, 12). In this largest study to date of 25(OH)D, PTH
247 and pregnancy outcomes, we report a two-fold increased prevalence ratio of elevated MAP in
248 women with functional vitamin D deficiency, defined by 25(OH)D $<$ 30 nmol/L and elevated

249 PTH, versus those with $25(\text{OH})\text{D} \geq 75$ nmol/L and normal PTH. This increase was present
250 with low vitamin D status only if PTH was concurrently elevated. A similar trend was
251 observed for SGA, although this was attenuated in regression models. Functional vitamin D
252 deficiency did not increase risks of gestational hypertension or preeclampsia, although
253 prevalence of preeclampsia was lowest, at 1.9%, in the reference group.

254 Our analysis follows earlier analyses by Scholl et al (11, 12), who reported that women with
255 functional vitamin D deficiency and calcium metabolic stress were two-three-fold more likely
256 to develop preeclampsia and SGA. Mixed parity women recruited to their multi-ethnic (86%
257 black or Hispanic) study were generally young (69% < 25 years of age) and of low
258 socioeconomic status (11, 12). We aimed to extend the concept of functional vitamin D
259 deficiency to a prospective cohort of well-characterized women in Northern Europe.

260 Participants in the SCOPE Ireland cohort were nulliparous with low risk pregnancies,
261 generally well-educated, mostly white and 89% were ≥ 25 years of age. In this cohort, as in
262 the literature (20), clear differences in both $25(\text{OH})\text{D}$ and PTH were evident between white
263 and other ethnicities, highlighting the need for explorations of vitamin D and the calcium
264 metabolic system that are specified by ethnicity. Thus, we restricted our current analysis to
265 white participants as the numbers of other ethnic groups were too small to analyze separately.

266 The prevalence of low vitamin D status in our cohort was higher than in many cohorts at
267 similar latitude, reflective of the lack of a mandated maternal supplementation policy,
268 analytical sensitivity and accuracy as well as inclement prevailing weather (16). Given that
269 we previously observed a reduction in the combined prevalence of preeclampsia+SGA with
270 $25(\text{OH})\text{D} \geq 75$ nmol/L (16) we chose this as the reference value for $25(\text{OH})\text{D}$ and defined
271 low $25(\text{OH})\text{D}$ status as < 30 nmol/L (7). There is a lack of clarity with regard to PTH
272 threshold levels in pregnancy, as PTH decreases in early gestation (21), reducing the
273 applicability of non-pregnancy thresholds. In this context, and given the population specific

274 nature of PTH (22), as well as well-documented, substantial analytical differences between
275 methods (23-27), we used a percentile cut-off, which although crude, may best capture
276 elevated PTH at a sample specific level. Additionally, we excluded the top 2.5% of PTH
277 values to minimise the risk of including undiagnosed cases of primary hyperparathyroidism,
278 which may have produced artificially elevated outcome risks. Although sample specific, there
279 may be some potential for misclassification of participants with use of a percentile cut-off as
280 a result of inherent inter-individual variation in PTH. Concentrations of PTH were lower in
281 our cohort than other studies (9, 11, 28, 29), but direct comparison is difficult; analytical
282 method, gestation and ethnicity influence PTH, as do specimen type (30) and BMI (31).

283 High PTH has been associated with blood pressure (32, 33) and cardiovascular risk indicators
284 (34), and there are a number of biologically plausible mechanisms through which the calcium
285 metabolic system may impact blood pressure. Belizán et al proposed that high PTH increases
286 intracellular calcium, triggering contraction of vascular smooth muscle cells and
287 vasoconstriction (35). Evidence of inter-play between the calcium metabolic system and
288 renin-angiotensin-aldosterone system is accumulating (36). Hemodynamics can be explored
289 through a range of measures and because a meta-analysis ($n = 60,599$) reported a second
290 trimester MAP > 90 mmHg to be predictive of preeclampsia (37) we investigated this
291 outcome in addition to gestational hypertension and preeclampsia. Although we report that
292 those with functional vitamin D deficiency were two-fold more likely to have elevated MAP
293 at 15 weeks' gestation, there was no increase in risk of gestational hypertension. Given that
294 25(OH)D, PTH and MAP were all measured at 15 weeks' gestation, this may reflect a time-
295 point specific occurrence. Elevated MAP and gestational hypertension are not mutually
296 inclusive outcomes; in this cohort 36% of those with elevated MAP at 15 weeks' developed
297 gestational hypertension and these outcomes may reflect different health profiles. Gestational

298 hypertension can occur at any stage in the second half of pregnancy and requires close
299 monitoring and treatment as clinically indicated (38).

300 Considering preeclampsia, the effect of vitamin D supplementation is not clear (19, 39).
301 However, combined vitamin D and calcium supplementation significantly reduced risk of
302 preeclampsia in three trials of 1,114 women (19). None of these trials compared each nutrient
303 individually versus combined supplementation and placebo. In a cohort in which 6%
304 developed preeclampsia, Scholl et al reported that participants with functional vitamin D
305 deficiency at < 20 weeks' gestation were three times more likely to develop preeclampsia
306 (11). Despite a substantially larger sample size, we did not replicate this effect, potentially
307 due to analytical differences and our application of a refined estimate of elevated PTH in a
308 'trimmed' distribution. The inclusion criteria for SCOPE, focusing on women with low risk
309 pregnancies, likely resulted in a different disease profile in those who developed
310 preeclampsia between the two cohorts; a relevant consideration given potential differences in
311 beneficial effects of vitamin D and calcium dependent on disease type and risk profile (40,
312 41). However, the lowest prevalence of preeclampsia occurred in those with $25(\text{OH})\text{D} \geq 75$
313 nmol/L with normal PTH, indicating a potential benefit of higher serum $25(\text{OH})\text{D}$, as
314 suggested by Aghajafari et al (4), particularly if PTH is not elevated.

315 With regards to SGA, fetal skeletal development represents a potential mechanism through
316 which perturbations in the maternal calcium metabolic system could predispose to SGA (42,
317 43) and interactions between vitamin D and calcium on fetal bone growth have been noted in
318 pregnant adolescents (44), a unique group due to dual growth requirements. Elevated blood
319 pressure can also predispose to SGA birth, both in association with and independently of
320 preeclampsia (45-47). Causative effects of vitamin D and calcium on fetal growth and SGA
321 have not been definitively established (39, 41, 48, 49). Observational evidence suggests
322 associations of both dairy intakes and PTH with fetal growth (50-52). A secondary analysis

323 of trial data in Gambian women, found no synergistic effect of calcium supplementation and
324 25(OH)D on fetal growth (53). There was little evidence of vitamin D deficiency in the
325 Gambian population who had low habitual calcium intakes, and these data may not be
326 applicable in our setting with prevalent vitamin D deficiency. In our analysis we did not
327 distinguish between SGA neonates born constitutionally small and those with fetal growth
328 restriction resulting in SGA, in whom outcomes may be worse (54).

329 To our knowledge, this is the largest study to investigate vitamin D and PTH in the context of
330 pregnancy and perinatal outcomes. Our data are strengthened by use of clinically defined
331 outcomes and the gold standard technique of CDC-accredited LC-MS/MS for measurement
332 of 25(OH)D. However, we did not have access to calcium intake data; this would have
333 enabled a more specific examination of calcium metabolic stress and perinatal outcomes,
334 although inclusion of PTH may capture some variation resulting from differences in calcium
335 intake. Harmonization and standardization of PTH analysis is required to define reference
336 ranges for pregnancy and for the purposes of this field, to better describe the 25(OH)D/PTH
337 relationship during gestation. In the absence of these data, the clinical significance of
338 elevated PTH is difficult to interpret.

339 In summary, prevalence of SGA was highest with functional vitamin D deficiency and we
340 have demonstrated evidence of functional vitamin D deficiency and elevated MAP, reflecting
341 the adverse impact of stress to the maternal calcium metabolic system in women at 15 weeks
342 of gestation. Though challenging in design and resource allocation, consideration should be
343 given to a priori inclusion of calcium intakes as well as quality-assured 25(OH)D and PTH
344 measurement in future studies of vitamin D and perinatal health.

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Table 1

Sample characteristics, PTH and 25(OH)D concentrations in the SCOPE Ireland pregnancy cohort ($n = 1754$)¹

Characteristic	%	25(OH)D		PTH	
		Concentration (nmol/L)	<i>P</i>	Concentration (pg/mL)	<i>P</i>
All participants		56.62 ± 25.8	-	7.84 (7.7, 8.0)	-
Ethnicity			< 0.001		0.001
White	97.7	57.11 ± 25.8		7.79 (7.6, 8.0)	
Other	2.3	35.54 ± 19.9		10.28 (8.5, 12.5)	
Age (years)			< 0.001		0.15
< 25	11.5	49.38 ± 27.8		7.66 (7.1, 8.2)	
≥ 25 - < 30	30.3	58.47 ± 26.5 ²		7.91 (7.6, 8.3)	
≥ 30 - < 35	45.0	57.88 ± 24.8 ²		7.69 (7.4, 8.0)	
≥ 35	13.2	54.33 ± 24.8		8.38 (7.8, 9.0)	
BMI (kg/m ²) ³			0.017		0.005 ⁴
< 18.5	1.2	54.39 ± 29.0		9.77 (7.1, 13.5)	
≥ 18.5 - < 25	58.4	58.14 ± 26.3		7.58 (7.4, 7.8)	
≥ 25 - < 30	28.1	55.30 ± 25.2		8.14 (7.8, 8.5)	
≥ 30	12.3	52.62 ± 24.3 ⁵		8.28 (7.7, 8.9)	
Smoking status ³			< 0.001		0.08
No	90.0	57.66 ± 25.8		7.90 (7.7, 8.1)	
Yes	10.0	47.23 ± 24.4		7.34 (6.8, 7.9)	
Alcohol consumption ³			0.10		0.47
No	83.6	56.17 ± 25.9		7.81 (7.6, 8.0)	
Yes	16.4	58.91 ± 25.5		8.00 (7.6, 8.5)	
Multi-nutrient supplementation ³			< 0.001		< 0.001
No	60.1	48.70 ± 23.8		8.15 (7.9, 8.4)	
Yes	39.9	68.54 ± 24.2		7.40 (7.1, 7.7)	
Season of entry ⁶			< 0.001		0.16

Winter	58.4	49.54 ± 24.6	7.96 (7.7, 8.2)	
Summer	41.6	66.57 ± 24.2	7.68 (7.4, 8.0)	
Elevated MAP ³			0.42	0.10
No	90.8	57.28 ± 25.6	7.73 (7.5, 7.9)	
Yes	9.2	55.61 ± 27.2	8.35 (7.6, 9.1)	
Gestational Hypertension			0.85	0.62
No	88.1	56.57 ± 25.8	7.82 (7.6, 8.0)	
Yes	11.9	56.93 ± 26.1	7.99 (7.4, 8.7)	
Preeclampsia			0.18	0.28
No	96.2	57.27 ± 25.8	7.77 (7.6, 8.0)	
Yes	3.8	52.97 ± 23.8	8.34 (7.3, 9.5)	
Small-for-gestational-age			0.003	0.16
No	89.4	57.74 ± 26.0	7.75 (7.5, 7.9)	
Yes	10.6	51.85 ± 23.3	8.20 (7.6, 8.9)	

¹PTH, geometric mean (95% CI) (all such values); 25(OH)D, mean ± SD (all such values). Differences assessed using independent samples t-test for binary variables and ANOVA for variables with multiple categories. Post-hoc analysis used Bonferroni correction. PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; SCOPE, Screening for Pregnancy Endpoints; MAP, mean arterial pressure.

² $P < 0.05$ compared to lowest group.

³At 15 week visit.

⁴Overall ANOVA significant but no significant between group differences after Bonferroni correction.

⁴ $P < 0.05$ compared to BMI ≥ 18.5 - < 25 kg/m².

⁶Winter: November through May and Summer: June through October.

Table 2PTH concentration by 25(OH)D category in the SCOPE Ireland pregnancy cohort ($n = 1754$)¹

25(OH)D (nmol/L)	%	PTH (pg/mL)
< 30	17.3	9.80 (9.2, 10.4) ^{3,4}
≥ 30 - < 40	13.6	8.50 (8.0, 9.1) ^{2,3,4}
≥ 40 - < 50	13.2	7.80 (7.3, 8.4) ^{2,4}
≥ 50 - < 75	30.7	7.54 (7.2, 7.9) ^{2,4}
≥ 75	25.2	6.78 (6.5, 7.1) ^{2,3}

¹PTH geometric mean (95% CI) (all such values).

Differences assessed using ANOVA. Overall *P*-trend for ANOVA, $P < 0.001$. Post hoc analysis used Bonferroni correction. PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; SCOPE, Screening for Pregnancy Endpoints.

² $P < 0.05$ compared with < 30 nmol/L.

³ $P < 0.05$ compared with 50 - 75 nmol/L.

⁴ $P < 0.05$ compared with ≥ 75 nmol/L.

Table 3

Association of 25(OH)D and PTH with elevated MAP in white participants of the SCOPE Ireland pregnancy cohort¹

25(OH)D (nmol/L)	PTH (percentile)	n	%	Elevated MAP (> 90 mmHg)	
				Unadjusted	Adjusted
≥ 75	—	441	9.3	Reference	Reference
< 30	—	283	12.0	1.29 (0.84, 1.99)	1.46 (0.90, 2.38) ²
—	≤ 80 th	1338	9.3	Reference	Reference
—	> 80 th	334	13.8	1.49 (1.08, 2.04)	1.28 (0.94, 1.75) ³
≥ 75	≤ 80 th	373	9.7	Reference	Reference
≥ 75	> 80 th	60	8.3	0.86 (0.35, 2.11)	0.89 (0.34, 2.28) ⁴
< 30	≤ 80 th	180	8.3	0.86 (0.49, 1.53)	0.91 (0.50, 1.65) ⁴
< 30	> 80 th	94	19.1	1.98 (1.18, 3.33)	1.83 (1.02, 3.27) ⁴

¹Values are PRs (95% CIs). Log-Poisson regression was used to examine the association between 25(OH)D and PTH thresholds (individually and in combination) and risk of elevated MAP. 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; MAP, mean arterial pressure; SCOPE, Screening for Pregnancy Endpoints.

²25(OH)D-MAP model adjusted for BMI, maternal age, university education and supplementation.

³PTH-MAP model adjusted for BMI, maternal age, smoking, supplementation, recreational walking and alcohol consumption.

⁴25(OH)D/PTH-MAP model adjusted for BMI, maternal age, smoking, supplementation and recreational walking.

Table 4

Associations of 25(OH)D and PTH with gestational hypertension, preeclampsia and SGA in white participants of the SCOPE Ireland pregnancy cohort¹

25(OH)D (nmol/L)	PTH (percentile)	n	Gestational Hypertension			Preeclampsia			Small-for-gestational-age		
			%	Unadjusted	Adjusted	%	Unadjusted	Adjusted	%	Unadjusted	Adjusted
≥ 75	—	441	14.3	Reference	Reference	2.3	Reference	Reference	7.3	Reference	Reference
< 30	—	283	12.7	0.89 (0.61, 1.30)	0.76 (0.51, 1.13) ²	4.9	2.18 (0.98, 4.84)	1.06 (0.42, 2.68) ³	13.1	1.80 (1.15, 2.82)	1.18 (0.70, 1.99) ⁴
—	≤ 80 th	1338	11.3	Reference	Reference	3.7	Reference	Reference	10.3	Reference	Reference
—	> 80 th	334	14.7	1.30 (0.96, 1.75)	1.18 (0.87, 1.59) ⁵	4.5	1.20 (0.68, 2.11)	1.13 (0.63, 2.00) ⁶	10.8	1.05 (0.74, 1.48)	1.00 (0.71, 1.43) ⁷
≥ 75	≤ 80 th	373	13.7	Reference	Reference	1.9	Reference	Reference	6.7	Reference	Reference
≥ 75	> 80 th	60	18.3	1.34 (0.74, 2.42)	1.24 (0.62, 2.50) ⁸	5.0	2.66 (0.71, 10.02)	2.36 (0.68, 8.22) ⁹	11.7	1.74 (0.79, 3.85)	1.72 (0.77, 3.88) ¹⁰
< 30	≤ 80 th	180	10.0	0.73 (0.44, 1.21)	0.61 (0.37, 1.02) ⁸	5.0	2.66 (1.01, 7.04)	1.32 (0.45, 3.87) ⁹	10.6	1.57 (0.89, 2.78)	0.97 (0.52, 1.81) ¹⁰
< 30	> 80 th	94	18.1	1.32 (0.80, 2.18)	1.00 (0.60, 1.67) ⁸	4.3	2.27 (0.68, 7.58)	1.17 (0.32, 4.20) ⁹	16.0	2.38 (1.31, 4.33)	1.53 (0.80, 2.93) ¹⁰

¹Values are RRs (95% CIs). Log-Poisson regression was used to examine the association between 25(OH)D and PTH (individually and in combination) and risk of gestational hypertension, preeclampsia and SGA. 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; SGA, small-for-gestational-age; SCOPE, Screening for Pregnancy Endpoints.

²25(OH)D-gestational hypertension model adjusted for BMI, smoking and recreational walking.

³25(OH)D-preeclampsia model adjusted for BMI, university education, and supplementation.

⁴25(OH)D-SGA model adjusted for university education, job status, supplementation and recreational walking.

⁵PTH-gestational hypertension model adjusted for BMI, smoking, supplementation, moderate exercise and infant sex.

⁶PTH-preeclampsia adjusted for BMI, university education, supplementation, moderate exercise and infant sex.

⁷PTH-SGA model adjusted for BMI, university education, job status, smoking and moderate exercise.

⁸25(OH)D/PTH-gestational hypertension model adjusted for BMI and recreational walking.

⁹25(OH)D/PTH-preeclampsia model adjusted for BMI, university education and supplementation.

¹⁰25(OH)D/PTH-SGA model adjusted for BMI, smoking, university education, job status, supplementation and recreational walking.

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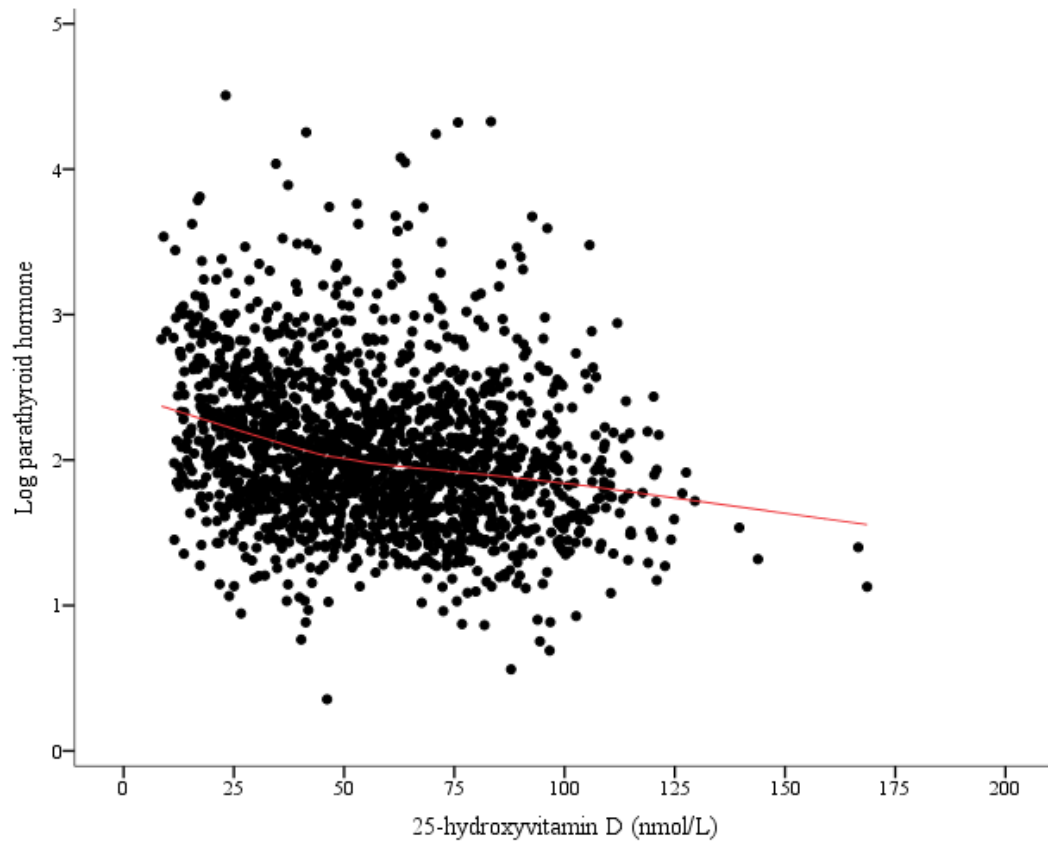


Figure 1. Scatterplot with Lowess curve of log parathyroid hormone and 25-hydroxyvitamin D in participants of the SCOPE Ireland pregnancy cohort ($n = 1754$).