

Title	Exploring the concept of functional vitamin D deficiency in pregnancy: impact of the interaction between 25-hydroxyvitamin D and parathyroid hormone on perinatal outcomes
Authors	Hemmingway, Andrea;Kenny, Louise C.;Malvisi, Lucio;Kiely, Mairead E.
Publication date	2018
Original Citation	Hemmingway, A., Kenny, L. C., Malvisi, L. and Kiely, M. E. (2018) 'Exploring the concept of functional vitamin D deficiency in pregnancy: impact of the interaction between 25-hydroxyvitamin D and parathyroid hormone on perinatal outcomes', The American Journal of Clinical Nutrition, 108(4), pp. 821-829. doi: 10.1093/ ajcn/nqy150
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://dx.doi.org/10.1093/ajcn/nqy150 - 10.1093/ajcn/nqy150
Rights	© 2018 American Society for Nutrition. Published by Oxford University Press. This is a pre-copyedited, author-produced version of an article accepted for publication in American Journal of Clinical Nutrition following peer review. The version of record, 108, Issue 4, 1 October 2018, Pages 821–829, is available online at: https://doi.org/10.1093/ajcn/nqy150
Download date	2025-06-01 08:11:56
Item downloaded from	https://hdl.handle.net/10468/7296



University College Cork, Ireland Coláiste na hOllscoile Corcaigh Exploring the concept of functional vitamin D deficiency in pregnancy: impact of the interaction between 25-hydroxyvitamin D and parathyroid hormone on perinatal outcomes

Andrea Hemmingway^{1,2}, Louise C Kenny³, Lucio Malvisi^{1,2}, Mairead E Kiely^{1,2*}

¹Cork Centre for Vitamin D and Nutrition Research, School of Food and Nutritional Sciences, University College Cork, Cork, Ireland (AH, LM, MEK)

²The Irish Centre for Fetal and Neonatal Translational Research (INFANT), University

College Cork, Cork, Ireland (AH, LCK, LM, MEK)

³Department of Women's and Children's Health, University of Liverpool, United Kingdom (LCK)

Authors' last names for indexing: Hemmingway, Kenny, Malvisi, Kiely

Disclaimers: The authors have no disclaimers.

*Corresponding author: Professor Mairead Kiely, Cork Centre for Vitamin D and Nutrition Research, Room 127, Level 1, Food Science Building, University College Cork, Western Road, Cork, Ireland, +353 214903394, <u>m.kiely@ucc.ie</u>

Sources of Support: This study and LM were supported by a grant to MEK from the European Commission for the Integrated Project ODIN (Food-based Solutions for Optimal Vitamin D Nutrition and Health through the Lifecycle, GA613977). The SCOPE Ireland pregnancy cohort study was funded by a grant to LCK from the Health Research Board of Ireland (CSA 02/2007). AH is supported by Science Foundation Ireland funding to MEK for COMBINE (INFANT/B3067), which is co-funded by the European Regional Development Fund (ERDF) under Ireland's European Structural and Investment Funds Programmes 2014-

2020. MEK is a principal investigator in the Science Foundation Ireland funded INFANT Research Centre (grant no. 12/RC/2272).

Short running head: Vitamin D, parathyroid hormone and pregnancy

Number of Tables: 4

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)

Background: Associations of vitamin D with perinatal outcomes are inconsistent and few
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.

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

9 **Results**: Mean \pm SD 25(OH)D was 56.6 \pm 25.8 nmol/L (22.7 \pm 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 $25(OH)D \ge 75$

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 \geq 75 nmol/L and normal PTH]. The adjusted prevalence ratio (PR) and

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

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

Accepted Manuscille

- 28 Introduction
- 29

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

53 Subjects and Methods

54 Study design and participants

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

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

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

83 Clinical definition of outcomes

84 Perinatal outcomes were precise and predefined (13), with participants followed prospectively throughout pregnancy and delivery. MAP was calculated from systolic and 85 diastolic BP measurements as diastolic BP + [(systolic BP – diastolic BP)/3], with MAP > 90 86 87 mmHg denoting elevated MAP. Gestational hypertension was defined as a systolic BP \geq 140 mmHg and/or diastolic BP \geq 90 mmHg on at least two occasions four hours apart after 20 88 weeks' of gestation but before the onset of labor. Preeclampsia was gestational hypertension 89 with either proteinuria (24 hour urinary protein \geq 300 mg or spot urine protein:creatinine ratio 90 \geq 30 mg/mmol creatinine or urine dipstick protein \geq 2+) or any multi-system complication of 91 preeclampsia. SGA birth was defined as a birth weight $< 10^{\text{th}}$ customized centile adjusted for 92 maternal height, booking weight and ethnicity as well as gestation at delivery and infant sex 93 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 $25(OH)D_2$ and $25(OH)D_3$.

99 Serum $25(OH)D_2$ and $25(OH)D_3$ 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 (Waters, Milford, USA). Four levels of serum-based NIST (National Institute of Standards 102 and Technology) certified quality assurance material (SRM 972) were used for method 103 104 validation while quality control materials assayed in parallel to all samples were purchased from Chromsystems (Munich, Germany). NIST calibrators (SRM 2972) were used 105 throughout the analysis. The limit of detection (LoD) for 25(OH)D₃ and 25(OH)D₂ was 0.31 106 107 and 0.44 nmol/L, respectively. The limit of quantitation (LoQ) for $25(OH)D_3$ and $25(OH)D_2$ was 1.03 and 1.43 nmol/L, respectively. Intra- and inter- assay CVs for both metabolites were 108 109 < 6% and < 5% respectively. The laboratory of the Cork Centre for Vitamin D and Nutrition Research is accredited by the CDC Vitamin D Standardization Certification program and 110 participates in the Vitamin D External Quality Assessment Scheme (DEQAS) (Charring 111 112 Cross Hospital, London, UK).

113 Serum PTH

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

126 Statistical analysis

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

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

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

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

- 175 **Results**
- 176

177 Descriptive results

Participant characteristics and 25(OH)D and PTH concentrations in the SCOPE Ireland 178 179 pregnancy cohort (n = 1754) are shown in **Table 1**. Mean \pm SD 25(OH)D concentration was $56.62 \pm 25.8 \text{ nmol/L} (22.7 \pm 10.3 \text{ ng/mL})$ and geometric mean (95% CI) PTH was 7.84 (7.7, 180 8.0) pg/mL [0.86 (0.85, 0.88) pmol/L]. This predominantly white (97.7%) cohort had a mean 181 \pm SD age of 30.5 \pm 4.5 years and BMI of 24.9 \pm 4.2 kg/m². At 15 weeks of gestation, 10.0% 182 and 16.4% of participants, respectively, reported current smoking and alcohol consumption. 183 Multi-nutrient supplements were consumed by 40% of participants and this was associated 184 with significantly higher 25(OH)D and lower PTH concentrations in consumers (P < 0.001185 186 for both). White ethnicity was also associated with higher 25(OH)D and lower PTH than other ethnicities ($P \le 0.001$ for both). Although season of entry was significantly associated 187 with 25(OH)D concentration (P < 0.001), PTH did not change with season. PTH increased 188 and 25(OH)D decreased with increasing BMI category at 15 weeks of gestation; however 189 post-hoc analysis revealed a significant decrease in 25(OH)D only in obese participants (BMI 190 \geq 30 kg/m²) compared to those with a normal BMI. PTH concentration was highest in the 191 small proportion of participants (1.2%) with a BMI < 18.5 kg/m². Mean arterial pressure was 192 79.1 ± 7.6 mmHg and 9.2% of participants had elevated MAP (> 90 mmHg). The prevalence 193 of gestational hypertension, preeclampsia and SGA in the cohort were 11.9%, 3.8% and 194 10.6%, respectively. 195

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

Table 2 depicts PTH concentrations across the distribution of 25(OH)D, showing that PTH

decreased significantly with increasing 25(OH)D (P < 0.001). Elevated PTH occurred more

- 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
- 17% were below 30 nmol/L, while 25% were had $25(OH)D \ge 75$ nmol/L. In the stratified
- analysis of 25(OH)D and PTH, the prevalence of functional vitamin D deficiency, defined as
- 206 $25(OH)D < 30 \text{ nmol/l plus PTH} > 80^{\text{th}}$ percentile, was much lower, at 5.5%, with 11.4%
- having a 25(OH)D < 50 nmol/L plus PTH > 80^{th} percentile.
- Associations of 25(OH)D, PTH and 25(OH)D/PTH groupings with elevated MAP are shown
- 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 \ge 75 nmol/L (P >
- 211 0.05). While there was an association of elevated PTH with elevated MAP [PR (95% CI);
- 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
- group [25(OH)D \ge 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 < 100
- 217 30 nmol/L if PTH was not elevated [adjusted PR (95% CI); 0.91 (0.50, 1.65)].
- **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
- elevated PTH and did not vary with 25(OH)D (both 18%, P > 0.05). Having functional
- vitamin D deficiency did not increase risk of gestational hypertension compared to the

- 225 reference, which was 25(OH)D ≥ 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 $25(OH)D \ge 75$ nmol/L and
- normal PTH, compared to 5.0% when $25(OH)D \ge 75$ nmol/L with elevated PTH (P > 0.05).
- In those with 25(OH)D < 30 nmol/L, the prevalence of preeclampsia did not differ depending
- on PTH status (P > 0.05). Occurrence of preeclampsia was not significantly increased (4.3%)
- versus 1.9%) with functional vitamin D deficiency compared to the reference group (P > 1
- 232 0.05) [adjusted RR (95% CI); 1.17 (0.32, 4.20)].

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

242

243 **Discussion**

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

PTH, versus those with $25(OH)D \ge 75$ nmol/L and normal PTH. This increase was present 249 with low vitamin D status only if PTH was concurrently elevated. A similar trend was 250 observed for SGA, although this was attenuated in regression models. Functional vitamin D 251 252 deficiency did not increase risks of gestational hypertension or preeclampsia, although prevalence of preeclampsia was lowest, at 1.9%, in the reference group. 253 254 Our analysis follows earlier analyses by Scholl et al (11, 12), who reported that women with functional vitamin D deficiency and calcium metabolic stress were two-three-fold more likely 255 to develop preeclampsia and SGA. Mixed parity women recruited to their multi-ethnic (86% 256 257 black or Hispanic) study were generally young (69% < 25 years of age) and of low socioeconomic status (11, 12). We aimed to extend the concept of functional vitamin D 258 deficiency to a prospective cohort of well-characterized women in Northern Europe. 259 260 Participants in the SCOPE Ireland cohort were nulliparous with low risk pregnancies, generally well-educated, mostly white and 89% were \geq 25 years of age. In this cohort, as in 261 the literature (20), clear differences in both 25(OH)D and PTH were evident between white 262 and other ethnicities, highlighting the need for explorations of vitamin D and the calcium 263 metabolic system that are specified by ethnicity. Thus, we restricted our current analysis to 264 265 white participants as the numbers of other ethnic groups were too small to analyze separately. The prevalence of low vitamin D status in our cohort was higher than in many cohorts at 266 similar latitude, reflective of the lack of a mandated maternal supplementation policy, 267 268 analytical sensitivity and accuracy as well as inclement prevailing weather (16). Given that we previously observed a reduction in the combined prevalence of preeclampsia+SGA with 269 $25(OH)D \ge 75$ nmol/L (16) we chose this as the reference value for 25(OH)D and defined 270 271 low 25(OH)D status as < 30 nmol/L (7). There is a lack of clarity with regard to PTH threshold levels in pregnancy, as PTH decreases in early gestation (21), reducing the 272 applicability of non-pregnancy thresholds. In this context, and given the population specific 273

274 nature of PTH (22), as well as well-documented, substantial analytical differences between methods (23-27), we used a percentile cut-off, which although crude, may best capture 275 elevated PTH at a sample specific level. Additionally, we excluded the top 2.5% of PTH 276 277 values to minimise the risk of including undiagnosed cases of primary hyperparathyroidism, which may have produced artificially elevated outcome risks. Although sample specific, there 278 may be some potential for misclassification of participants with use of a percentile cut-off as 279 a result of inherent inter-individual variation in PTH. Concentrations of PTH were lower in 280 our cohort than other studies (9, 11, 28, 29), but direct comparison is difficult; analytical 281 method, gestation and ethnicity influence PTH, as do specimen type (30) and BMI (31). 282 High PTH has been associated with blood pressure (32, 33) and cardiovascular risk indicators 283 (34), and there are a number of biologically plausible mechanisms through which the calcium 284 285 metabolic system may impact blood pressure. Belizán et al proposed that high PTH increases intracellular calcium, triggering contraction of vascular smooth muscle cells and 286 vasoconstriction (35). Evidence of inter-play between the calcium metabolic system and 287 renin-angiotensin-aldosterone system is accumulating (36). Hemodynamics can be explored 288 through a range of measures and because a meta-analysis (n = 60,599) reported a second 289 290 trimester MAP > 90 mmHg to be predictive of preeclampsia (37) we investigated this outcome in addition to gestational hypertension and preeclampsia. Although we report that 291 those with functional vitamin D deficiency were two-fold more likely to have elevated MAP 292 293 at 15 weeks' gestation, there was no increase in risk of gestational hypertension. Given that 25(OH)D, PTH and MAP were all measured at 15 weeks' gestation, this may reflect a time-294 point specific occurrence. Elevated MAP and gestational hypertension are not mutually 295 296 inclusive outcomes; in this cohort 36% of those with elevated MAP at 15 weeks' developed gestational hypertension and these outcomes may reflect different health profiles. Gestational 297

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

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

With regards to SGA, fetal skeletal development represents a potential mechanism through 315 316 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 pregnant adolescents (44), a unique group due to dual growth requirements. Elevated blood 318 pressure can also predispose to SGA birth, both in association with and independently of 319 320 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 associations of both dairy intakes and PTH with fetal growth (50-52). A secondary analysis 322

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

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

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

Acknowledgements

The authors' responsibilities were as follows: MEK designed the research, is the ODIN and COMBINE grant-holder and guarantor of the manuscript; LCK is the SCOPE PI globally and has overall responsibility for the SCOPE Ireland pregnancy cohort; AH conducted the PTH sample analysis and data analysis; LM conducted statistical analysis and drafted the statistical methodology; AH and MEK wrote the manuscript and MEK was responsible for the final content. 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

- 1. Leffelaar ER, Vrijkotte TG, van Eijsden M. Maternal early pregnancy vitamin D status in relation to fetal and neonatal growth: results of the multi-ethnic Amsterdam Born Children and their Development cohort. Br J Nutr 2010;104:108-17.
- 2. Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. J Nutr 2007;137:447-52.
- 3. Saraf R, Morton SM, Camargo CA Jr., Grant CC. Global summary of maternal and newborn vitamin D status a systematic review. Matern Child Nutr 2016;12:647-68.
- 4. Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. BMJ 2013;346:f1169.
- 5. Thorne-Lyman A, Fawzi WW. Vitamin D during pregnancy and maternal, neonatal and infant health outcomes: a systematic review and meta-analysis. Paediatr Perinat Epidemiol 2012;26:75-90.
- 6. Brannon PM, Picciano MF. Vitamin D in pregnancy and lactation in humans. Annu Rev Nutr 2011;31:89-115.
- 7. Institute of Medicine. Dietary reference intakes for Calcium and Vitamin D. Washington (DC): National Academies Press, 2011.
- 8. Kiely M, Hemmingway A, O'Callaghan KM. Vitamin D in pregnancy: current perspectives and future directions. Ther Adv Musculoskelet Dis 2017;9:145-54.
- Hamilton SA, McNeil R, Hollis BW, Davis DJ, Winkler J, Cook C, Warner G, Bivens B, McShane P, Wagner CL. Profound vitamin D deficiency in a diverse group of women during pregnancy living in a sun-rich environment at latitude 32°N. Int J Endocrinol 2010:917428.
- 10. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev 2001;22:477-501.
- 11. Scholl TO, Chen X, Stein TP. Vitamin D, secondary hyperparathyroidism, and preeclampsia. Am J Clin Nutr 2013;98:787-93.
- 12. Scholl TO, Chen X, Stein TP. Maternal calcium metabolic stress and fetal growth. Am J Clin Nutr 2014;99:918-25.
- 13. Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, McCowan LM, Simpson NA, Dekker GA, Roberts CT, et al. Early pregnancy prediction of preeclampsia

in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study. Hypertension 2014;64:644-52.

- 14. Engstrom JL, Paterson SA, Doherty A, Trabulsi M, Speer KL. Accuracy of self-reported height and weight in women: an integrative review of the literature. J Midwifery Womens Health 2003;48:338-45.
- 15. McCowan L, Stewart AW, Francis A, Gardosi J. A customised birthweight centile calculator developed for a New Zealand population. Aust N Z J Obstet Gynaecol 2004;44:428-31.
- 16. Kiely ME, Zhang JY, Kinsella M, Khashan AS, Kenny LC. Vitamin D status is associated with uteroplacental dysfunction indicated by pre-eclampsia and small-forgestational-age birth in a large prospective pregnancy cohort in Ireland with low vitamin D status. Am J Clin Nutr 2016;104:354-61.
- Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911-30.
- 18. Zou G. A Modified Poisson Regression Approach to Prospective Studies with Binary Data. Am J Epidemiol 2004;159:702-6.
- 19. De-Regil LM, Palacios C, Lombardo LK, Peña-Rosas JP. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev 2016;1:CD008873.
- 20. Gutiérrez OM, Farwell WR, Kermah D, Taylor EN. Racial differences in the relationship between vitamin D, bone mineral density, and parathyroid hormone in the National Health and Nutrition Examination Survey. Osteoporos Int 2011;22:1745-53.
- 21. Møller UK, Streym S, Mosekilde L, Heickendorff L, Flyvbjerg A, Frystyk J, Jensen LT, Rejnmark L. Changes in calcitropic hormones, bone markers and insulin-like growth factor I (IGF-I) during pregnancy and postpartum: a controlled cohort study. Osteoporos Int 2013;24:1307-20.
- 22. Souberbielle JC, Brazier F, Piketty ML, Cormier C, Minisola S, Cavalier E. How the reference values for serum parathyroid hormone concentration are (or should be) established? J Endocrinol Invest 2017;40:241-56.
- 23. Worth GK, Vasikaran SD, Retallack RW, Musk AA, Gutteridge DH. Major methodspecific differences in the measurement of intact parathyroid hormone: studies in patients with and without chronic renal failure. Ann Clin Biochem 2004;41:149-54.
- 24. Cantor T, Yang Z, Caraiani N, Ilamathi E. Lack of comparability of intact parathyroid hormone measurements among commercial assays for end-stage renal disease patients: implication for treatment decisions. Clin Chem 2006;52:1771-6.

- 25. Souberbielle JC, Boutten A, Carlier MC, Chevenne D, Coumaros G, Lawson-Body E, Massart C, Monge M, Myara J, Parent X, et al. Inter-method variability in PTH measurement: implication for the care of CKD patients. Kidney Int 2006;70:345-50.
- 26. Sukumar D, Shapses S, Partridge NC, Schneider S. Intervariability among serum intact parathyroid hormone assays: a need for standardization. Osteoporos Int 2008;19:1805-6.
- 27. Cavalier E, Delanaye P, Vranken L, Bekaert AC, Carlisi A, Chapelle JP, Souberbielle JC. Interpretation of serum PTH concentrations with different kits in dialysis patients according to the KDIGO guidelines: importance of the reference (normal) values. Nephrol Dial Transplant 2012;27:1950-6.
- Morley R, Carlin JB, Pasco JA, Wark JD. Maternal 25-hydroxyvitamin D and parathyroid hormone concentrations and offspring birth size. J Clin Endocrinol Metab 2006;91:906-12.
- 29. Haddow JE, Neveux LM, Palomaki GE, Lambert-Messerlian G, Canick JA, Grenache DG, Lu J. The relationship between PTH and 25-hydroxy vitamin D early in pregnancy. Clin Endocrinol (Oxf) 2011;75:309-14.
- 30. Glendenning P, Laffer LL, Weber HK, Musk AA, Vasikaran SD. Parathyroid hormone is more stable in EDTA plasma than in serum. Clin Chem 2002;48:766-7.
- 31. Aloia JF, Feuerman M, Yeh JK. Reference range for serum parathyroid hormone. Endocr Pract 2006;12:137-44.
- 32. Kim H, Chung YE, Jung SC, Im H, Yang SY, Kim DY, Jeong E, Kim B, Park SK. Independent associations of circulating 25-hydroxyvitamin D and parathyroid hormone concentrations with blood pressure among Koreans: The Korea National Health and Nutrition Examination Survey (KNHANES), 2009-2010. Calcif Tissue Int 2013;93:549-55.
- 33. Snijder MB, Lips P, Seidell JC, Visser M, Deeg DJ, Dekker JM, van Dam RM. Vitamin D status and parathyroid hormone levels in relation to blood pressure: a population-based study in older men and women. J Intern Med 2007;261:558-65.
- 34. Bosworth C, Sachs MC, Duprez D, Hoofnagle AN, Ix JH, Jacobs DR Jr., Peralta CA, Siscovick DS, Kestenbaum B, de Boer IH. Parathyroid hormone and arterial dysfunction in the multi-ethnic study of atherosclerosis. Clin Endocrinol (Oxf) 2013;79:429-36.
- 35. Belizán JM, Villar J, Repke J. The relationship between calcium intake and pregnancyinduced hypertension: up-to-date evidence. Am J Obstet Gynecol 1988;158:898-902.
- 36. Vaidya A, Brown JM, Williams JS. The renin-angiotensin-aldosterone system and calcium-regulatory hormones. J Hum Hypertens 2015;29:515-21.
- 37. Cnossen JS, Vollebregt KC, de Vrieze N, ter Riet G, Mol BW, Franx A, Khan KS, van der Post JA. Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meta-analysis. BMJ 2008;336:1117-20.

- National Institute for Health and Clinical Excellence. Hypertension in Pregnancy: The Management of Hypertensive Disorders During Pregnancy. London: RCOG Press 2010. Updated 2017.
- 39. Roth DE, Leung M, Mesfin E, Qamar H, Watterworth J, Papp E. Vitamin D supplementation during pregnancy: state of the evidence from a systematic review of randomised trials. BMJ 2017;359:j5237.
- 40. Dror DK. Vitamin D status during pregnancy: maternal, fetal, and postnatal outcomes. Curr Opin Obstet Gynecol 2011;23:422-6.
- 41. Hofmeyr GJ, Lawrie TA, Atallah AN, Duley L, Torloni MR. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. Cochrane Database Syst Rev 2014;6:CD001059.
- 42. Dror DK, King JC, Fung EB, Van Loan MD, Gertz ER, Allen LH. Evidence of associations between feto-maternal vitamin D status, cord parathyroid hormone and bone-specific alkaline phosphatase, and newborn whole body bone mineral content. Nutrients 2012;4:68-77.
- 43. Beltrand J, Alison M, Nicolescu R, Verkauskiene R, Deghmoun S, Sibony O, Sebag G, Lévy-Marchal C. Bone mineral content at birth is determined both by birth weight and fetal growth pattern. Pediatr Res 2008;64:86-90.
- 44. Young BE, McNanley TJ, Cooper EM, McIntyre AW, Witter F, Harris ZL, O'Brien KO. Maternal vitamin D status and calcium intake interact to affect fetal skeletal growth in utero in pregnant adolescents. Am J Clin Nutr 2012;95:1103-12.
- 45. Block-Abraham DM, Adamovich D, Turan OM, Doyle LE, Blitzer MG, Baschat AA. Maternal blood pressures during pregnancy and the risk of delivering a small-for-gestational-age neonate. Hypertens Pregnancy 2016;35:350-60.
- 46. Wikström AK, Gunnarsdottir J, Nelander M, Simic M, Stephansson O, Cnattingius S. Prehypertension in pregnancy and risks of small for gestational age infant and stillbirth. Hypertension 2016;67:640-6.
- 47. Anderson NH, Sadler LC, Stewart AW, Fyfe EM, McCowan LM. Independent risk factors for infants who are small for gestational age by customised birthweight centiles in a multi-ethnic New Zealand population. Aust N Z J Obstet Gynaecol 2013;53:136-42.
- 48. Harvey NC, Holroyd C, Ntani G, Javaid K, Cooper P, Moon R, Cole Z, Tinati T, Godfrey K, Dennison E, et al. Vitamin D supplementation in pregnancy: a systematic review. Health Technol Assess 2014;18:1-190.
- 49. Buppasiri P, Lumbiganon P, Thinkhamrop J, Ngamjarus C, Laopaiboon M, Medley N. Calcium supplementation (other than for preventing or treating hypertension) for improving pregnancy and infant outcomes. Cochrane Database Syst Rev 2015;2:CD007079.

- Chang SC, O'Brien KO, Nathanson MS, Caulfield LE, Mancini J, Witter FR. Fetal femur length is influenced by maternal dairy intake in pregnant African American adolescents. Am J Clin Nutr 2003;77:1248-54.
- 51. Mannion CA, Gray-Donald K, Koski KG. Association of low intake of milk and vitamin D during pregnancy with decreased birth weight. CMAJ 2006;174:1273-7.
- 52. Brunvand L, Quigstad E, Urdal P, Haug E. Vitamin D deficiency and fetal growth. Early Hum Dev 1996;45:27-33.
- 53. Prentice A, Jarjou LM, Goldberg GR, Bennett J, Cole TJ, Schoenmakers I. Maternal plasma 25-hydroxyvitamin D concentration and birthweight, growth and bone mineral accretion of Gambian infants. Acta Paediatr 2009;98:1360-2.
- 54. Alberry M, Soothill P. Management of fetal growth restriction. Arch Dis Child Fetal Neonatal Ed 2007;92:F62-7.

Table 1

$\frac{\text{cohort } (n=1754)^1}{2}$	

		25(OH)D		PTH	
Characteristic	%	Concentration (nmol/L)	Р	Concentration (pg/mL)	Р
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)	
\geq 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)	
\geq 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

58.4	49.54 ± 24.6		7.96 (7.7, 8.2)	
41.6	66.57 ± 24.2		7.68 (7.4, 8.0)	
		0.42		0.10
90.8	57.28 ± 25.6		7.73 (7.5, 7.9)	
9.2	55.61 ± 27.2		8.35 (7.6, 9.1)	
		0.85		0.62
88.1	56.57 ± 25.8		7.82 (7.6, 8.0)	Κ.
11.9	56.93 ± 26.1		7.99 (7.4, 8.7)	
		0.18		0.28
96.2	57.27 ± 25.8		7.77 (7.6, 8.0)	
3.8	52.97 ± 23.8		8.34 (7.3, 9.5)	
		0.003	3-	0.16
89.4	57.74 ± 26.0		7.75 (7.5, 7.9)	
10.6	51.85 ± 23.3		8.20 (7.6, 8.9)	
	58.4 41.6 90.8 9.2 88.1 11.9 96.2 3.8 89.4 10.6	58.4 49.54 ± 24.6 41.6 66.57 ± 24.2 90.8 57.28 ± 25.6 9.2 55.61 ± 27.2 88.1 56.57 ± 25.8 11.9 56.93 ± 26.1 96.2 57.27 ± 25.8 3.8 52.97 ± 23.8 89.4 57.74 ± 26.0 10.6 51.85 ± 23.3	58.4 49.54 ± 24.6 41.6 66.57 ± 24.2 0.42 90.8 57.28 ± 25.6 9.2 55.61 ± 27.2 0.85 88.1 56.57 ± 25.8 11.9 56.93 ± 26.1 0.18 96.2 57.27 ± 25.8 3.8 52.97 ± 23.8 0.003 89.4 57.74 ± 26.0 10.6 51.85 ± 23.3	58.4 49.54 ± 24.6 $7.96 (7.7, 8.2)$ 41.6 66.57 ± 24.2 $7.68 (7.4, 8.0)$ 0.42 0.42 90.8 57.28 ± 25.6 $7.73 (7.5, 7.9)$ 9.2 55.61 ± 27.2 $8.35 (7.6, 9.1)$ 0.85 0.85 88.1 56.57 ± 25.8 $7.82 (7.6, 8.0)$ 11.9 56.93 ± 26.1 $7.99 (7.4, 8.7)$ 0.18 0.18 96.2 57.27 ± 25.8 $7.77 (7.6, 8.0)$ 3.8 52.97 ± 23.8 $8.34 (7.3, 9.5)$ 0.003 0.003 89.4 57.74 ± 26.0 $7.75 (7.5, 7.9)$ 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.

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

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

PTH concentration b	y 25(OH)D	category in the SCOPE Ire	eland pregnancy cohort (n =	= 1754) ¹
---------------------	-----------	---------------------------	-----------------------------	----------------------

25(OH)D (nmol/L)	%	PTH (pg/mL)
< 30	17.3	9.80 (9.2, 10.4) ^{3,4}
\ge 30 - < 40	13.6	8.50 (8.0, 9.1) ^{2,3,4}
\ge 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

			Elevated MAP (> 90 mmHg)					
25(OH)D (nmol/L)	PTH (percentile)	n	%	Unadjusted	Adjusted			
≥75	_	441	9.3	Reference	Reference			
< 30		283	12.0	1.29 (0.84, 1.99)	$1.46 (0.90, 2.38)^2$			
—	$\leq 80^{th}$	1338	9.3	Reference	Reference			
—	$> 80^{th}$	334	13.8	1.49 (1.08, 2.04)	$1.28 (0.94, 1.75)^3$			
≥75	$\leq 80^{\text{th}}$	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	$\leq 80^{th}$	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

			Gestational Hypertension				Preeclampsia			Small-for-gestational-age		
25(OH)D (nmol/L)	PTH (percentile)	n	%	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)	$1.18 (0.70, 1.99)^4$	
_	$\leq 80^{th}$	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	$\leq 80^{th}$	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	$\leq 80^{\text{th}}$	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	$> 80^{\text{th}}$	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.



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