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The role of parathyroid hormone during pregnancy on the relationship between maternal vitamin D deficiency and fetal growth restriction: a prospective birth cohort study

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Abstract

Previous studies have shown conflicting findings regarding the relationship between maternal vitamin D deficiency (VDD) and fetal growth restriction (FGR). We hypothesised that parathyroid hormone (PTH) may be an underlying factor relevant to this potential association. In a prospective birth cohort study, descriptive statistics were evaluated for the demographic characteristics of 3407 pregnancies in the second trimester from three antenatal clinics in Hefei, China. The association of the combined status of vitamin D and PTH with birth weight and the risk of small for gestational age (SGA) was assessed by a multivariate linear and binary logistic regression. We found that declined status of 25-hydroxyvitamin D is associated with lower birth weight (for moderate VDD: adjusted $\beta = -49{\cdot}4$ g, 95 % CI −91·1, −7·8, $P < 0{\cdot}05$; for severe VDD: adjusted $\beta = -79{\cdot}8$ g, 95 % CI −127·2, −32·5, $P < 0{\cdot}01$), as well as ascended levels of PTH (for elevated PTH: adjusted $\beta = -44{\cdot}5$ g, 95 % CI −82·6, −6·4, $P < 0{\cdot}05$). Compared with the non-VDD group with non-elevated PTH, pregnancies with severe VDD and elevated PTH had the lowest neonatal birth weight (adjusted $\beta = -124{\cdot}7$ g, 95 % CI −194·6, −54·8, $P < 0{\cdot}001$) and the highest risk of SGA (adjusted risk ratio (RR) = 3·36, 95 % CI 1·41, 8·03, $P < 0{\cdot}01$). Notably, the highest risk of less Ca supplementation was founded in severe VDD group with elevated PTH (adjusted RR = 4·67, 95 % CI 2·78, 7·85, $P < 0{\cdot}001$). In conclusion, elevated PTH induced by less Ca supplementation would further aggravate the risk of FGR in pregnancies with severe VDD through impaired maternal Ca metabolism homoeostasis.

Key words: **Pregnancy: Vitamin D deficiency: Parathyroid hormone: Birth weight: Fetal growth restriction**

Birth weight has been considered a proxy for fetal growth and development and is associated with future risk of adult metabolic diseases, particularly in low birth weight[1]. It is well known that small for gestational age (SGA) newborns have an increased risk of neonatal and infant mortality, short stature, neurocognitive impairment and other outcomes, including chronic disease in adulthood[2–6].

Vitamin D (VD) is an essential hormone for Ca homoeostasis and bone mineralisation of the developing fetus[7]. Previous observational studies have suggested that lower maternal 25-hydroxyvitamin D (25(OH)D) concentrations were associated with lower birth weight[8,9], others have not found such an association[10,11]. Moreover, a recent meta-analysis of thirteen randomised controlled trials indicated that maternal VD supplementation significantly increased birth weight and attenuated the risk of low birth weight and SGA compared with the no intervention group[12]. Despite these findings, a limited number of randomised controlled trials could not completely

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; FGR, fetal growth restriction; PTH, parathyroid hormone; RR, risk ratio; SGA, small for gestational age; VD, vitamin D; VDD, vitamin D deficiency.

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confirm the positive effect of VD supplementation during pregnancy on fetal growth[13–16]. These conflicting results suggest that underlying factors relevant to this potential association need to be discovered.

Evidence from the results of the 'Mamma & Bambino' cohort and a meta-analysis suggested that decreased birth weight may be affected by vitamin D receptor gene polymorphisms and mutated alleles, but the specific effect still needs to be further corroborated[17]. Another under-recognised biological factor that may contribute to this conflicting literature is parathyroid hormone (PTH). Indeed, as the key upstream hormonal regulator of VD, PTH stimulates the kidneys to produce 1,25-$(OH)_2D_3$ and enhances the tubular reabsorption of Ca. It also activates osteoblasts, which stimulate the transformation of pre-osteoclasts into mature osteoclasts[9,18,19]. Moreover, the 25(OH)D concentration above an individual threshold could provide maximal PTH suppression[20]. Thus, VD helps maintain appropriate serum Ca concentrations by regulating the release of PTH to enhance Ca absorption. It is possible that the combined serum status of VD and PTH during pregnancy is a better determinant of fetal growth.

Therefore, we hypothesised that elevated PTH may further increase the risk of adverse fetal development in mothers with lower maternal 25(OH)D concentrations. In this study, we aimed to estimate the role of PTH during pregnancy on the relationship between maternal vitamin D deficiency (VDD) and fetal growth restriction (FGR) in a prospective cohort study from China.

Methods

Study population

From March 2015 to December 2017, a total of 4768 consecutive healthy pregnancies in the second trimester (gestational age at 14–27 weeks) were recruited from three different antenatal clinics in Hefei (Hefei First People's Hospital, Hefei Maternal and Child Care Hospital and the First Affiliated Hospital of Anhui Medical University), China. The exclusion criteria of the study participants were multiple gestation (*n* 113), assisted conception (*n* 108), drug or alcohol abuse (*n* 90) and residence outside of Hefei (*n* 251). We followed up the participants until delivery and excluded serious pregnancy complications (e.g. gestational hypertension and preeclampsia, moderate and severe eclampsia, acute or chronic liver or renal diseases and chronic diseases, *n* 276), abortion (*n* 85), stillbirth (*n* 51), birth defection (congenital anomalies, genetic abnormalities or skeletal dysplasia, *n* 143) and loss to follow-up (birth outcome information not obtained, *n* 244). Finally, we obtained the full data of 3407 mother–infant pairs (Fig. 1). The Ethics Committee of the Anhui Medical University (reference number 2015002; date of approval 22 April 2015) approved the study and all participants provided written informed consent before enrolment.

Data collection

Face-to-face interviews at enrolment were used to obtain information about the socio-economic and demographic characteristics, health status and lifestyle relevant to VD status. Socio-economic and demographic characteristics include maternal age, gestational age at enrolment, maternal education level, place of residence, parity and household income level. Health status includes anthropometric measurements (height and weight) and medical history. Variables on lifestyle relevant to VD status include the frequency of consuming milk, eggs, Ca and vitamin D supplements. Complications of pregnancy were obtained from medical records. The pre-pregnancy BMI was calculated based on self-reported pre-pregnancy weight and measured height[21]. The season of blood sample collection at enrolment was defined as two periods of the year: winter/spring (December–May) and summer/autumn (June–November). Gestational weight gain was calculated based on the pre-pregnancy weight and the weight recorded at delivery or the last prenatal visit before delivery.

The birth outcome indexes were obtained from medical records, and birth weight was measured by study nurses. The accuracy of the scales used to measure birth weight was checked using standard weights at the beginning of the study. Gestational age (in completed weeks) was calculated based on the history of the last menstrual period. SGA was defined based on the method developed by Mikolajczyk *et al.*[22] as a birth weight lower than the 10th percentile for the gestational age-specific value of the study cohort (*n* 3407).

Serum 25-hydoxyvitamin D, parathyroid hormone and calcium

At enrolment, fasting maternal venous blood samples were drawn from participants after 15 min of rest and were processed by study nurses. Serum samples were stored to −80°C freezers for long-term storage until analysis. Circulating 25(OH)D is the best biomarker of VD status because it represents VD obtained from both dietary sources and UV skin synthesis[23]. The quantification of serum 25(OH)D concentration was measured using commercial radioimmunoassay kits (DiaSorin Stillwater). The intra-assay and inter-assay coefficients of variation were 8·8 and 11·1 %, respectively. Serum concentrations of intact PTH were determined using a direct chemiluminescence immunoassay (ADVIA Centaur XP; Siemens Healthcare Diagnostics), with intra- and inter-assay coefficients of variations of 4·2 and 11·9 %, respectively. The levels of ionised Ca in the serum sample were determined by flame atomic absorption spectrometry (using a Zeiss AAS-3 spectrometer with deuterium background correction). The accuracy of the method was verified using certified reference material (Hum Asy Control 2, Randox) and was 95 %.

Second-trimester 25(OH)D and Ca concentrations were measured in 3407 pregnancies. We randomly selected half of the total sample (*n* 1703) and assayed for PTH concentrations. However, 167 samples failed to detect PTH; therefore, we obtained 1536 serum PTH concentrations. Attrition analyses showed that the distributions of socio-economic and demographic characteristics, health status, lifestyle, birth outcomes and VD levels in 1536 pregnant women with PTH measurements did not differ from those of all participants.

Serum 25(OH)D concentrations were categorised into groups as severe VDD (<30 nmol/l), moderate VDD (≥30–<50 nmol/l) and non-VDD (≥50 nmol/l)[24]. In the absence of pregnancy-specific reference ranges, elevated PTH levels (≥16·7 ng/l) were

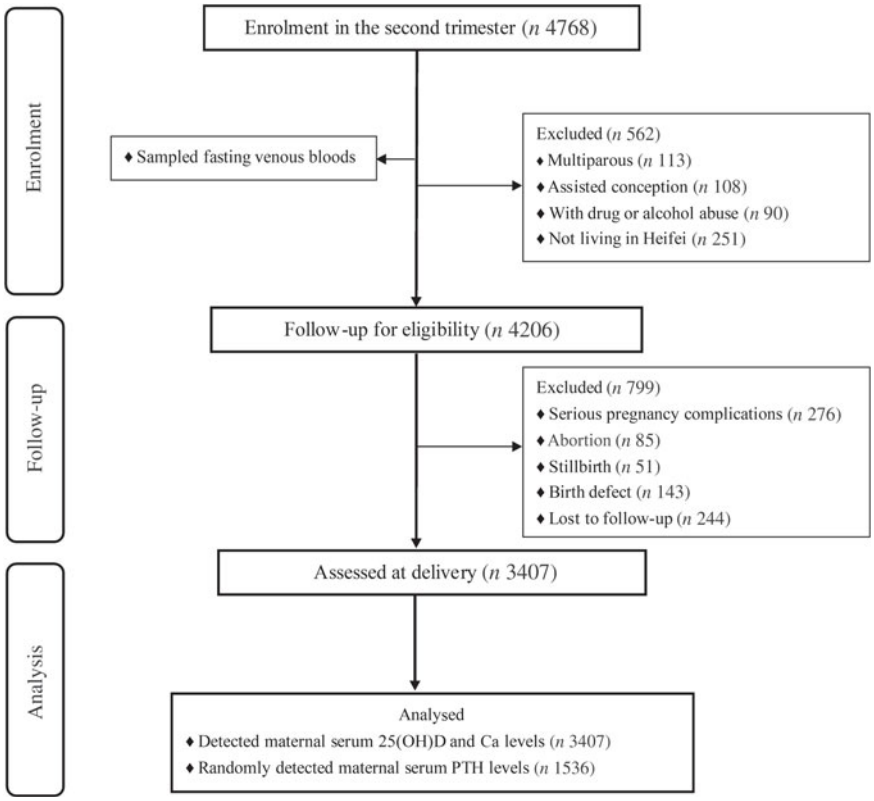

Fig. 1. Flow diagram of study participants enrolment, follow-up and analysis. 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

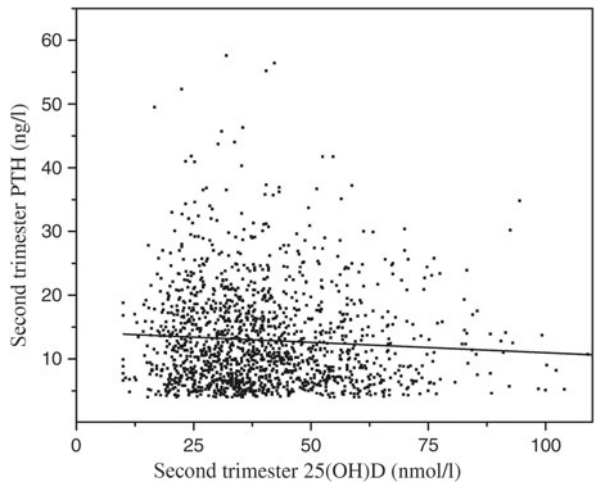

Fig. 2. Scatter plot between parathyroid hormone (PTH) and 25-hydroxyvitamin D (25(OH)D) concentrations in the second trimester (*n* 1536).

defined by a concentration that exceeded the upper reference limit for 75th percentiles (P_{75}). Non-elevated PTH levels were defined as concentrations within the upper reference limit for P_{75} (<16·7 ng/l).

Statistical analysis

The socio-economic and demographic characteristics and the laboratory statistics of 3407 mother–infant pairs are shown as means and standard deviations or as percentages. The associations of serum 25(OH)D and Ca supplement frequency with PTH were tested with linear regression or binary logistic analysis.

The individual relationships of serum 25(OH)D or PTH status with birth weight and SGA risk were evaluated by multivariate linear regression analysis and binary logistic regression after adjustment for confounders, including gestational age at enrolment, household income, parity, pre-pregnancy BMI, gestational diabetes, gestational weight gain, VD supplement, Ca supplement, milk supplement, sunshine exposure, blood sampling season, maternal anaemia and fetal sex. To identify the unique contribution of each factor of maternal 25(OH)D and PTH status, the other factor was controlled in multivariate linear regression models.

Multiple linear regression and binary logistic regression were accordingly used to calculate the association of the combined status of VD and PTH in the second trimester with birth weight and the risk of SGA, with adjustment for confounders. Similarly, the association of the combined status of VD and PTH with serum Ca and Ca supplement frequency was analysed by multiple linear regression and binary logistic regression. Multiple linear regression analysis generated β-coefficients and 95 % CI. Likewise, binary logistic regression analysis generated adjusted risk ratios (RR) and 95 % CI. Sensitivity analyses were performed to confirm the consistency of the results for different percentile cut-offs for elevated PTH. We performed all analyses using IBM SPSS version 21.0 software (IBM Corp.).

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Results

Socio-economic, demographic and laboratory characteristics of the study population

A total of 3407 mother–infant pairs were included in the study. The average age of the study population was 29·2 (SD 4·4) years, and the gestational age upon entry into the study was 24·2 (SD 2·4) weeks. The mean concentrations of serum 25(OH)D and Ca were 39·9 (SD 16·3) nmol/l and 1·52 (SD 0·19) μmol/l, respectively. The percentages of the women who had severe, moderate VDD and non-VDD were 29·4, 46·9 and 23·7 %, respectively. The mean serum PTH concentration was 12·9 (SD 7·6) ng/l, and the threshold concentration of P_{75} was 16·7 ng/l. The percentage of less Ca supplementation (<3 times/week) was 25·1 %. The mean birth weight was 3402·4 (SD 397·6) g, and the gestational age at delivery was 39·1 (SD 1·2) weeks (Table 1).

Associations of vitamin D and calcium supplement frequency with parathyroid hormone levels

Associations of VD and Ca supplement frequency with PTH levels during the second trimester of pregnancy are shown in online Supplementary Table S1. Serum PTH concentrations ($\beta = 1{\cdot}69$ ng/l, 95 % CI 0·63, 2·74, $P < 0{\cdot}01$) and the risk of elevated PTH (RR = 1·66, 95 % CI 1·20, 2·31, $P < 0{\cdot}01$) were significantly increased in the severe VDD group compared with the non-VDD group. There was a weak but statistically significant inverse association (r −0·068, $P = 0{\cdot}008$) between 25(OH)D and PTH concentration (Fig. 2). Similarly, PTH concentrations ($\beta = 1{\cdot}09$ ng/l, 95 % CI 0·24, 1·94, $P < 0{\cdot}05$) and the risk of elevated PTH (RR = 1·41, 95 % CI 1·08, 1·85, $P < 0{\cdot}05$) were significantly increased in pregnancies with a lower frequency of Ca supplementation (<3 times/week) compared with the reference group (≥5 times/week).

Table 1. Characteristics of the study population, enrolment through to birth (*n* 3407)
(Mean values and standard deviations; numbers and percentages)

Characteristics	Mean or *n*	SD or %
Maternal		
Gestational week at enrolment (weeks)	24·2	2·4
Maternal age (years)	29·2	4·4
Pre-pregnancy BMI (kg/m^2)	21·3	2·8
Living in urban area	3124	91·7
Education (high school or more)	2920	85·7
Household income < 6000 yuan/month	341	10·0
Nulliparous	1963	57·6
Gestational diabetes	605	17·8
Anaemia	684	20·1
GWG (kg)	12·5	3·9
Vitamin D supplement < 3 (times/week)	3293	96·7
Ca supplement < 3 (times/week)	856	25·1
Milk supplement < 3 (times/week)	1423	41·8
Sunshine exposure < 30 (min/d)	2791	81·9
Blood sampling season (December–May)	1720	50·5
Entry Ca (μmol/l)	1·52	0·19
Entry 25(OH)D (nmol/l)	39·9	16·3
Vitamin D status		
<30 nmol/l	1003	29·4
≥30–<50 nmol/l	1597	46·9
≥50 nmol/l	807	23·7
Entry PTH (ng/l)	12·9	7·6
PTH levels*		
P_{25}, P_{50}, P_{75} (ng/l)	7·0, 11·0, 16·7	
Newborns		
Gestational age at delivery (weeks)	39·1	1·2
Male	1772	52·0
Birth weight (g)	3402·4	397·6
SGA	301	8·8

GWG, gestational weight gain; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; SGA, small for gestational age.
* *n* was 1536.

Individual association of maternal vitamin D deficiency and elevated parathyroid hormone with fetal growth

The individual relationships of serum VD or PTH status with neonatal birth weight and the risk of SGA are presented in Table 2. In the multiple linear regression model, the neonatal birth weight in pregnancies with severe or moderate VDD was, respectively, −79·8 g (95 % CI −127·2, −32·5, $P < 0{\cdot}01$) and −49·4 g (95 % CI −91·1, −7·8, $P < 0{\cdot}05$) lower than that in non-VDD pregnancies after adjustments were made for potential confounders. Similarly, the neonatal birth weight of pregnancies with elevated PTH decreased by 41·8 g (95 % CI −80·3, −3·2, $P < 0{\cdot}05$) compared with that of pregnancies with non-elevated PTH. However, the risk of SGA significantly increased only in pregnancies with severe VDD (adjusted RR = 2·24, 95 % CI 1·22, 4·10, $P < 0{\cdot}01$) but not elevated PTH compared with the reference group.

Neonatal birth weight: the influence of the combined status of maternal serum 25-hydroxyvitamin D and parathyroid hormone

Table 3 shows the role of PTH during the second trimester on the relationship between maternal VDD and neonatal birth weight. Compared with the reference group (non-VDD and non-elevated PTH levels), pregnancies with severe or moderate VDD were significantly associated with lower birth weight, regardless of PTH status. Neonatal birth weight progressively and significantly decreased (adjusted $\beta = -20{\cdot}8$ g, 95 % CI −32·0, −9·5) across the different combined statuses of descending VD and ascending PTH. Infants whose mothers had severe VDD and elevated PTH had the lowest birth weight (adjusted $\beta = -124{\cdot}7$ g, 95 % CI −194·6, −54·8, $P < 0{\cdot}001$). Among pregnancies with severe VDD, the neonatal birth weight of pregnancies with non-elevated PTH significantly increased (adjusted $\beta = 67{\cdot}5$ g, 95 % CI 1·2, 133·7) compared with pregnancies with elevated PTH. In addition, among pregnancies with severe or moderate VDD, the birth weight of pregnancies with non-elevated PTH significantly increased (adjusted $\beta = 42{\cdot}3$ g, 95 % CI 17·2, 67·5) compared with pregnancies with elevated PTH.

The risk of small for gestational age: the influence of the combined status of serum 25-hydroxyvitamin D and parathyroid hormone

In the multiple regression model for the association of the combined status of serum 25(OH)D and PTH with the risk of SGA (Table 4), the risk of SGA (adjusted RR = 1·23, 95 % CI 1·07, 1·41) progressively and significantly increased across the different combined statuses of descending VD and

Table 2. Individual association of vitamin D deficiency or elevated parathyroid hormone (PTH) with fetal growth*
(Mean values and standard deviations; β-coefficients and 95 % confidence intervals; numbers and percentages; risk ratios (RR))

		Birth weight (g)					SGA				
Serum status	*n*	Mean	SD	β	95 % CI	*P*	*n*	%	RR	95 % CI	*P*
25(OH)D status											
<30 nmol/l	1003	3374·6	407·6	−79·8	−127·2, −32·5	0·001	99	9·9	2·24	1·22, 4·10	0·009
≥30–<50 nmol/l	1597	3403·2	400·7	−49·4	−91·1, −7·8	0·020	141	8·8	1·66	0·96, 2·87	0·070
≥50 nmol/l	807	3417·3	390·4	Ref	Ref		61	7·6	Ref	Ref	
PTH levels											
$<P_{75}$	1153	3407·4	377·8	Ref	Ref	Ref	87	7·5	Ref	Ref	Ref
$\geq P_{75}$	383	3383·4	354·5	−44·5	−82·6, −6·4	0·022	33	8·6	1·16	0·73, 1·84	0·532

SGA, small for gestational age; 25(OH)D, 25-hydroxyvitamin D; Ref, reference.
* Adjusted for gestational age at delivery, gestational age at enrolment, maternal age at enrolment, household income, nulliparous, pre-pregnancy BMI, gestational diabetes, gestational weight gain, vitamin D supplement, Ca supplement, milk supplement, sunshine exposure, blood sampling season, sex, anaemia, vitamin D status (for PTH levels) or PTH status (for 25(OH)D status).

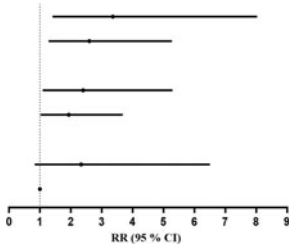
Table 3. Association of combined status of maternal serum 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH) with infant birth weight*
(Mean values and standard deviations; β-coefficients and 95 % confidence intervals)

Groups	*n*	Mean (g)	SD	β (g)	95 % CI	*P*
VD < 30 nmol/l						
PTH ≥ P_{75}	131	3364·2	351·8	−124·7	−194·6, −54·8	<0·001
PTH < P_{75}	314	3388·3	383·5	−80·8	−134·6, −27·1	0·003
≥30–<50 nmol/l						
PTH ≥ P_{75}	180	3388·6	350·4	−92·6	−154·1, −31·1	0·003
PTH < P_{75}	552	3402·4	386·2	−51·8	−98·7, −4·9	0·031
VD ≥ 50 nmol/l						
PTH ≥ P_{75}	72	3405·4	372·5	−49·3	−133·1, 34·4	0·248
PTH < P_{75}	287	3437·7	353·8	Ref	Ref	

VD, vitamin D; Ref, reference.
* Adjusted for gestational age at delivery, gestational age at enrolment, maternal age at enrolment, household income, nulliparous, pre-pregnancy BMI, gestational diabetes, gestational weight gain, VD supplement, Ca supplement, milk supplement, sunshine exposure, blood sampling season, sex and anaemia.

Table 4. Influence of combined status of maternal serum 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH) on the risk of small for gestational age*
(Numbers and percentages; risk ratios (RR) and 95 % confidence intervals)

Groups	*n*	*n*	%		RR	95 % CI	*P*
VD < 30 nmol/l							
PTH ≥ P_{75}	131	12	9·2		3·36	1·41, 8·03	0·006
PTH < P_{75}	314	28	8·9		2·60	1·28, 5·27	0·008
≥30–<50 nmol/l							
PTH ≥ P_{75}	180	15	8·3		2·40	1·09, 5·29	0·031
PTH < P_{75}	552	44	8·0		1·93	1·02, 3·68	0·044
VD ≥ 50 nmol/l							
PTH ≥ P_{75}	72	6	8·3		2·33	0·83, 6·50	0·107
PTH < P_{75}	287	15	5·2		Ref	Ref	Ref



VD, vitamin D; Ref, reference.
* Adjusted for gestational age at delivery, gestational age at enrolment, maternal age at enrolment, household income, nulliparous, pre-pregnancy BMI, gestational diabetes, gestational weight gain, VD supplement, Ca supplement, milk supplement, sunshine exposure, blood sampling season, sex and anaemia.

ascending PTH. Pregnancies with severe VDD and elevated PTH had a significantly higher risk of SGA (adjusted RR = 3·36, 95 % CI 1·41, 8·03, $P < 0·01$). Among pregnancies with severe or moderate VDD, the SGA risk of pregnancies with non-elevated PTH significantly decreased (adjusted RR = 0·68, 95 % CI 0·25, 0·98) compared with that of pregnancies with elevated PTH.

Serum calcium metabolism and calcium supplement: the association of the combined status of serum 25-hydroxyvitamin D and parathyroid hormone

Serum Ca concentrations progressively and significantly increased (adjusted $\beta = 0·007$ μmol/l, 95 % CI 0·001, 0·014) across the different combined statuses of descending VD and ascending PTH. Pregnancies with severe VDD and elevated

Table 5. Associations of combined status of vitamin D (VD) and parathyroid hormone (PTH) with serum calcium metabolism and frequency of calcium supplementation during pregnancy*
(*β*-Coefficients and 95 % confidence intervals; numbers and percentages; risk ratios (RR))

		Ca (μmol/l)			Less frequency of Ca supplement†				
Groups	*n*	*β*	95 % CI	*P*	*n*	%	RR	95 % CI	*P*
VD < 30 nmol/l									
PTH ≥ P_{75}	131	0·050	0·011, 0·089	0·012	50	38·2	4·67	2·78, 7·85	<0·001
PTH < P_{75}	314	0·029	−0·001, 0·059	0·061	101	32·2	3·07	1·98, 4·74	<0·001
≥30–<50 nmol/l									
PTH ≥ P_{75}	180	0·021	−0·014, 0·055	0·235	46	25·6	2·31	1·41, 3·80	0·001
PTH < P_{75}	552	0·020	−0·007, 0·046	0·148	116	21·0	1·80	1·19, 2·71	0·005
VD ≥ 50 nmol/l									
PTH ≥ P_{75}	72	0·013	−0·035, 0·060	0·601	12	16·7	1·40	0·68, 2·86	0·363
PTH < P_{75}	287	Ref	Ref	Ref	38	13·2	Ref	Ref	Ref

Ref, reference.
* Adjusted for gestational age at delivery, gestational age at enrolment, maternal age at enrolment, household income, nulliparous, pre-pregnancy BMI, gestational diabetes, gestational weight gain, VD supplement, milk supplement, sunshine exposure, blood sampling season, sex and anaemia.
† Less frequency of Ca supplement: Ca supplement <3 times/week.

PTH had significantly higher serum Ca concentrations (adjusted $\beta = 0{\cdot}050$ (95 % CI 0·011, 0·089) μmol/l, $P < 0{\cdot}05$) compared with non-VDD and non-elevated PTH pregnancies. Consistently, the proportion of less Ca supplement frequency (<3 times/week) during pregnancy progressively and significantly increased (adjusted RR = 1·34, 95 % CI 1·23, 1·46) across the different combined statuses of descending VD and ascending PTH. Pregnancies with severe VDD and elevated PTH had a significantly higher prevalence (38·2 %) of lower Ca intake frequency (<3 times/week) compared with non-VDD and non-elevated PTH pregnancies (Table 5).

Sensitivity analyses: the consistency of analysis results for different percentile cut-offs for elevated parathyroid hormone

Sensitivity analyses were performed to confirm the consistency of the analysis results for different percentile cut-offs for elevated PTH. We found that the associations of the combined status of VDD and elevated PTH (≥P_{80}) with birth weight, SGA, maternal Ca and Ca supplementation frequency were similar to the aforementioned analysis results in which elevated PTH was defined as PTH levels ≥75th percentiles. Pregnancies with severe VDD and elevated PTH (≥P_{80}) had the lowest neonatal birth weight and the highest risk of SGA. Consistently, the highest serum Ca levels and the highest proportion of lower Ca supplementation were observed in pregnancies with severe VDD and elevated PTH (≥P_{80}) (online Supplementary Tables S2 and S3).

Discussion

In this study, we evaluated the effect of the combined serum status of VD and PTH during pregnancy on FGR in a large sample and prospective birth cohort. The results showed that severe VDD in the second trimester could independently lead to decreased neonatal birth weight and an increased risk of SGA. Unlike VD, elevated PTH was significantly associated with decreased neonatal birth weight but not an increased risk of SGA. However, elevated PTH might further aggravate the risk of FGR for pregnant women with VDD, especially those with severe VDD. This result highlights the role of PTH on the association between maternal VD and fetal growth and implicates PTH as a potential factor that may be relevant to both conflicting studies to date regarding maternal VD and fetal growth and clinical trials of VD supplementation in pregnancy.

Several previous reports have suggested an association between VD insufficiency in pregnancy and FGR. A recent meta-analysis[25] of thirty-one studies demonstrated that pregnant women with low serum 25(OH)D levels had an increased risk of low birth weight and SGA infants. Conversely, in two cohort studies[10,26], there were no associations between maternal VD status and infant birth weight. Moreover, the U-shaped association between 25(OH)D and FGR has also been reported in several observational studies[27,28]. In this context of conflicting findings, our study reinforces the independent association between maternal VD status during the second trimester and FGR in a cohort of 3407 mother–infant pairs and strictly controls a large number of confounding variables. However, it remains unclear whether VDD exerts a causal effect on FGR, which warrants further investigation in the context of a randomised trial.

It was a distinct advantage that our study simultaneously evaluated the individual and combined role of maternal PTH on FGR, which has been largely ignored in nearly all previous studies. There are few reports relevant to the association between PTH and fetal growth. A previous study[29] found that maternal serum PTH at delivery was inversely related to the crown-heel length among thirty pregnant women. In contrast, an observational study[30] of 374 pregnancies demonstrated that maternal PTH concentrations at 28–32 weeks were weakly positively associated with neonatal birth weight. However, these results should be interpreted with caution because of the small study sample or the absence of adjustments for confounders. In this large sample study with strict control of confounders, elevated PTH was significantly associated with decreased neonatal birth weight but not an increased risk of SGA, which was partly supported by another recent large sample study[31]. However, the most valuable finding in our study was the combined effect of PTH and VD during pregnancy on FGR.

To date, few studies have reported the combined effect of maternal VD and PTH on fetal development. As there is a lack

of clarity with regard to PTH threshold levels in pregnancy, elevated PTH was usually defined according to a percentile cut-off. A status of VDD (<30 nmol/l) and elevated PTH was identified as functional VDD[32,33]. Scholl *et al.*[30] reported that mothers with functional VDD were 2- to 3-fold more likely to develop SGA and that neonatal birth weight was reduced by 102–128 g. Hemmingway *et al.*[31] observed in a large sample cohort study that the prevalence of SGA was highest ($P < 0{\cdot}05$) in mothers with functional VDD; however, the SGA risk (adjusted RR 1·53; 95 % CI 0·80, 2·93) was attenuated in a fully adjusted model. A similar trend was observed in our study, such that infants whose mothers with functional VDD (severe VDD with elevated PTH) had the lowest birth weight (reduced by 127·7 g) and the highest SGA risk (increased by 3·19-fold). The gradual effects across the different combined statuses of descending VD and ascending PTH on FGR were also observed in our study. Although ethnic differences in the sample population between our study and the above two studies were evident, our study strongly identified the enormous adverse effect of functional VDD during pregnancy on fetal growth.

At present, the mechanism through which functional VDD during pregnancy was linked to FGR remains unclear. VD has well-defined classical functions related to Ca metabolism[34,35]. Ca metabolic stress, in which adverse effects of VDD are mediated through a functional impact on the Ca metabolic system, was considered as the critical mechanism by Scholl *et al.*[33] and Hemmingway *et al.*[31]. However, in their studies, they did not evaluate serum Ca or Ca supplementation. Uniquely, in our study, we simultaneously assessed Ca supplementation during pregnancy and maternal serum Ca concentrations. We found that compared with the reference group, Ca concentrations significantly increased in mothers with functional VDD (severe VDD with elevated PTH) but not in mothers with severe VDD and non-elevated PTH, which was considered a sign of stress to Ca metabolism of functional VDD[36]. Consistently, the proportion of lower Ca supplement frequency (<3 times/week) during pregnancy is highest with functional VDD. Maternal Ca homoeostasis is associated with fetal Ca homoeostasis to support fetal development[37]. Therefore, our finding suggested that elevated PTH levels induced by less Ca supplementation might further aggravate FGR for pregnancies with severe VDD through impaired maternal Ca metabolism homoeostasis.

Strengths and limitations

Our study was a prospective cohort study. The number of participants was relatively large, and a large number of confounding factors relevant to VD status and fetal development were strictly controlled. Our findings are strengthened by the consistency of the analysis results for different fetal outcome indexes (birth weight and SGA), the sensitivity analyses for different percentile cut-offs for elevated PTH and the evaluation of maternal Ca metabolism stress (Ca supplement and serum Ca). However, we should acknowledge that there are some limitations in our study. First, as a lack of clarity elevated PTH threshold concentrations in pregnancy, a percentile cut-off is used to define elevated PTH, which might lead to misclassification. Therefore, the clinical significance of elevated PTH is difficult to interpret. The harmonisation and standardisation of PTH analysis are required to define reference ranges for pregnancy in future research in this context. Second, we did not evaluate fetal Ca metabolism and other markers related to fetal growth, although the inclusion of maternal serum Ca concentrations may partly reflect fetal Ca metabolism status. The mechanism through which maternal functional VDD was linked to fetal growth still has not been illuminated.

Conclusions

In conclusion, maternal PTH may be a potential risk factor relevant to the association between maternal VD and fetal growth. Elevated PTH levels induced by less Ca supplementation might further aggravate fetal growth in pregnant women with severe VDD through impaired maternal Ca metabolism homoeostasis. Functional VDD in the second trimester, defined as VDD (<30 nmol/l) with elevated PTH (>P_{75}), predicted the enormous adverse effect on fetal growth. Treating VDD in pregnancy (and not only Ca supplements) may contribute to optimal fetal growth. Evidence from our study may warrant further investigation to illuminate the clinical significance in the context of a randomised trial.

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Supplementary material

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