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PEDIATRIC ORIGINAL ARTICLE

Deficit of vitamin D in pregnancy and growth and overweight in the offspring

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BACKGROUND: Maternal vitamin D status during fetal development may influence offspring growth and risk of obesity; however, evidence in humans is limited.
OBJECTIVE: To investigate whether maternal circulating 25-hydroxyvitamin D3 (25(OH)D3) concentration in pregnancy is associated with offspring prenatal and postnatal growth and overweight.
METHODS: Plasma 25(OH)D3 concentration was measured in pregnant women (median weeks of gestation 14.0, range 13.0–15.0) from the INMA (INfancia y Medio Ambiente) cohort (Spain, 2003–2008) ($n = 2358$). Offspring femur length (FL), biparietal diameter (BPD), abdominal circumference (AC) and estimated fetal weight (EFW) were evaluated at 12, 20 and 34 weeks of gestation by ultrasound examinations. Fetal overweight was defined either as AC or as EFW $\geqslant$ 90th percentile. Child's anthropometry was recorded at ages 1 and 4 years. Rapid growth was defined as a weight gain *z*-score of >0.67 from birth to ages 6 months and 1 year. Age- and sex-specific *z*-scores for body mass index (BMI) were calculated at ages 1 and 4 years (World Health Organization referent); infant's overweight was defined as a BMI *z*-score $\geqslant$ 85th percentile.
RESULTS: We found no association of maternal 25(OH)D3 concentration with FL and a weak inverse association with BPD at 34 weeks. Maternal deficit of 25(OH)D3 (< 20 ng ml^{-1}) was associated with increased risk of fetal overweight defined as AC $\geqslant$ 90th percentile (odds ratio (OR) = 1.50, 95% confidence interval (CI): 1.01–2.21; $P = 0.041$) or either as EFW $\geqslant$ 90th percentile (OR = 1.47, 95% CI: 1.00–2.16; $P = 0.046$). No significant associations were found with rapid growth. Deficit of 25(OH)D3 in pregnancy was associated with an increased risk of overweight in offspring at age 1 year (OR = 1.42, 95% CI: 1.02–1.97; $P = 0.039$); however, the association was attenuated at age 4 years (OR = 1.19, 95% CI: 0.83–1.72; $P = 0.341$).
CONCLUSIONS: Vitamin D deficiency in pregnancy may increase the risk of prenatal and early postnatal overweight in offspring. Clinical trials are warranted to determine the role of vitamin D in the early origins of obesity.

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INTRODUCTION

Growth before birth and during the early postnatal period may have long-term effects on health in the life course including risk of obesity. *In utero*, the offspring is entirely dependent on the mother for an adequate supply of 25-hydroxyvitamin D (25(OH)D),[1] and an inadequate maternal vitamin D status exposes the offspring to insufficiency during potentially critical phases of development. Vitamin D plays an important role in calcium homeostasis, bone remodeling and muscle function,[2] and the lack of vitamin D during pregnancy may result in impaired bone development, fetal growth and adverse effects on musculoskeletal health and obesity in the offspring.[3–6]

Vitamin D affects lypolysis and adipogenesis in humans via modulation of adipocyte calcium signaling,[7–9] and therefore may have a role in the origins of obesity. In adults, vitamin D deficiency is emerging as a risk factor for components of the metabolic syndrome including abdominal obesity.[10,11] Evidence of the influence of vitamin D status on obesity in childhood and adolescence is also growing, although the cause–effect relationship remains unclear. Cross-sectional[12–14] and prospective[15] studies conducted in children and adolescents have reported associations of lower circulating vitamin D concentrations with greater body mass index (BMI) and adiposity outcomes. Furthermore, BMI and the prevalence of obesity vary as a function of month of birth;[16,17] greater adiposity is found for men and women born in winter–spring, and this may partially reflect the effect of fetal exposure to low vitamin D during certain periods of gestation. Few studies have investigated the role of vitamin D status *in utero* in the early origins of obesity. In a small cohort of UK children, offspring weight, fat mass and lean mass at age 9 years were not associated with maternal 25(OH)D concentrations measured in late pregnancy.[18] However, a follow-up study in Indian children found maternal 25(OH)D in late pregnancy to be negatively associated with 9.5-year fat percentage and positively

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with fat-free percentage.[19] Recently, in a large UK prospective cohort study, Crozier *et al.*[20] have reported lower maternal circulating 25(OH)D concentration in late pregnancy to be associated with lower dual-energy X-ray absorptiometry-assessed fat mass in the offspring at birth but with greater fat mass at ages 4 and 6 years.

Given the high prevalence of vitamin D deficiency among childbearing women, and increasing rates of childhood obesity worldwide, the study of growth and obesity-related outcomes in children born to mothers with an inadequate vitamin D status deserves further investigation with noteworthy clinical importance and public health implications. We therefore conducted a large prospective study to investigate the association of maternal circulating 25(OH)D3 concentration with offspring prenatal and postnatal growth outcomes and risk of rapid growth and overweight during infancy in a population-based cohort study.

MATERIALS AND METHODS

Study population

The INMA—INfancia y Medio Ambiente—(Environment and Childhood) Project[21] is a prospective population-based cohort study in Spain that recruited a total of 2644 women who fulfilled the inclusion criteria (that is, $\geqslant 16$ years of age, intention to deliver at the reference hospital, no problems of communication, singleton pregnancy and no assisted conception) during the first prenatal visit between November 2003 and February 2008 in four geographical areas: Valencia (39°N latitude, $n=855$), Sabadell (41°N latitude, $n=657$), Gipuzkoa (42°N latitude, $n=638$) and Asturias (43°N latitude, $n=494$). The Ethical Committees of the Institutions involved in the project approved the study and written informed consent was obtained from the parents of all children. For the present study, we analyzed data of circulating concentration of 25(OH)D3 measured in maternal blood samples collected in pregnancy, offspring fetal biometry and anthropometry measures recorded at birth and at ages 1 and 4 years. Circulating concentration of 25(OH)D3 was measured in 2489 mothers (94% of initially recruited), and fetal biometry was available for 2358 participants (89%) who were eligible for the present study (Figure 1). Complete data on maternal 25(OH)D3 concentration, confounders and fetal biometry were available on 2221 mother–child pairs (84% of initially included); on exposure, confounders and offspring anthropometry at age 1 year on 1468 mother–child pairs (56% of initially included); and on exposure, confounders and offspring anthropometry at age 4 years on 1344 mother–child pairs (51% of initially recruited) (Figure 1).

Assessment of maternal circulating 25(OH)D3 concentration

A single maternal blood specimen was drawn during pregnancy (median weeks of gestation 14.0, range 13.0–15.0). Samples were processed immediately and stored from −70 to −80 °C until analysis. Plasma concentrations of 25(OH)D3 were quantified by high-performance liquid chromatography method by using a BIO-RAD kit (BIO-RAD Laboratories GmbH, Munchen, Germany) according to Clinical and Laboratory Standard Institute protocols.[22] Detection limit was 5 ng ml^{-1}, and interassay coefficient of variation was 4.5%. The assay was validated by German Programmes of External Evaluation of Quality (DGKLRFB-Referencezinstutuk fur Bionalytik), and results were satisfactory in 100% of the cases.

Fetal biometry and anthropometry at birth

All ultrasound measurements were performed at routinely scheduled antenatal care visits by specialized obstetricians. For each participant we acquired from 2 to 7 valid ultrasound examinations conducted between 7 and 42 weeks of gestation. An early crown–rump length measurement was also obtained and it was used for pregnancy dating. Gestational age was established using crown–rump length when the difference with the age based on the self-reported last menstrual period was 7 days or more. Women for whom this difference exceeded 3 weeks were removed from the study to avoid possible bias. Fetal characteristics assessed were biparietal diameter (BPD), femur length (FL) and abdominal circumference (AC). All measurements were performed in mm using transabdominal ultrasound examination. Linear-mixed models were used separately in each area of study to obtain longitudinal growth curves for BPD, AC, FL as well as for estimated fetal weight (EFW) estimated by using the Hadlock algorithm.[23,24] Box–Cox transformations were used on these outcomes in order to normalize them. Each transformed outcome was modeled as a polynomial of gestational age in days until degree 3. Models were adjusted for the following constitutional factors known to affect fetal growth: maternal height, age, parity, ethnicity and pre-pregnancy weight, father's height and fetal sex.[25–27] These constitutional factors and their interactions with days of gestation were tested with the likelihood ratio test ($P<0.05$) through a forward-selection procedure. The length of time between ultrasound examinations was used to model the correlation structure for intrasubject errors. Gestational age, sex,

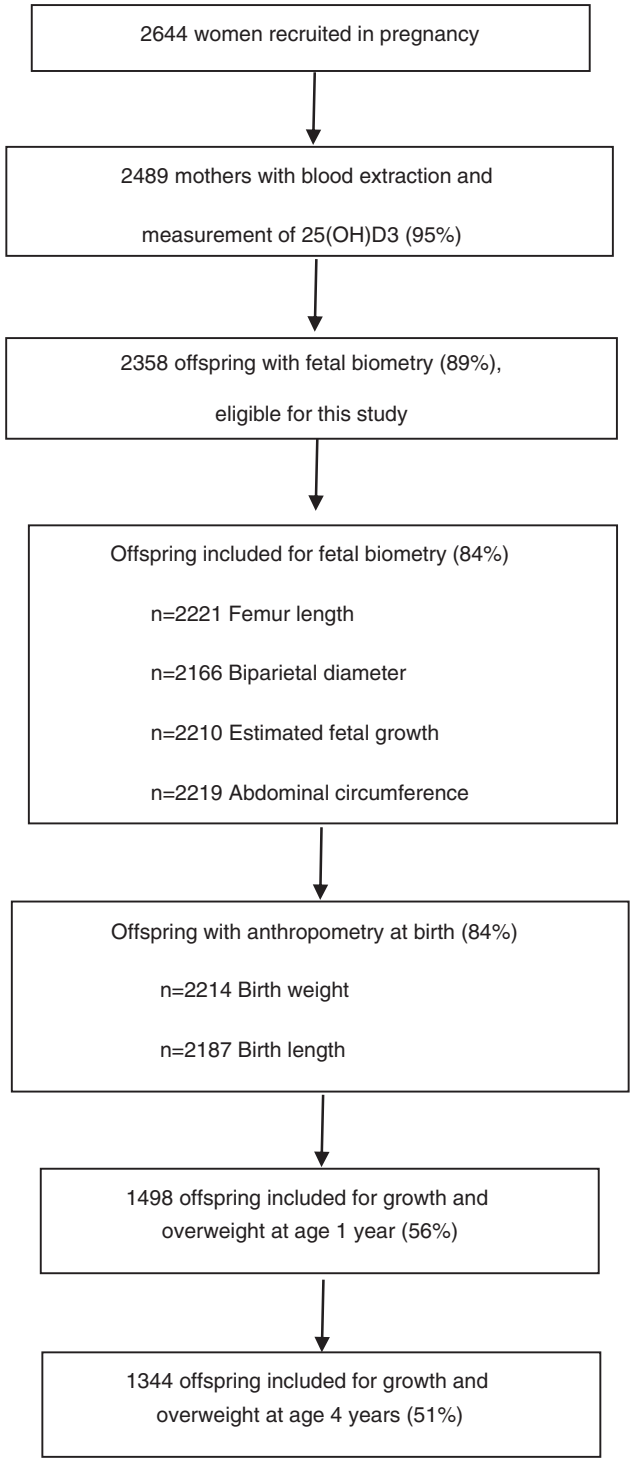

Figure 1. Flowchart of the study population.

parity, ethnicity and dummy variables identifying mothers who had ultrasound examinations spaced too closely in time to show changes in fetal growth parameters were used to estimate variance (heteroscedasticity). Random effects of the curves of constitutional factors versus growth on intercept, slope (days of gestation) or both were considered and tested with the likelihood ratio test ($P < 0.05$). Goodness of fit was assessed by consideration of the normality and independence of the residuals.

Mean, s.d. and predictions for weeks 12, 20 and 34 conditioned on the nearest measurement were obtained from fetal curves and were used for calculating unconditional and conditional s.d. scores.[28] The unconditional s.d. score at a certain time point describe the size of a fetus at that time. The s.d. score at the end of a time interval conditioned on the value at the starting point describes the growth experienced in this interval.[28–30] Unconditional *z*-scores were obtained at 12, 20 and 34 weeks of gestation. Fetal overweight was defined either as AC or as EFW $\geqslant$ 90th percentile.[31]

The offspring anthropometric measures at birth were weight in g and birth length in cm. Birth weight was measured by the midwife attending the birth, whereas birth length was measured by a nurse when the newborn arrived at the hospital ward within the first 12 h of life.

Offspring postnatal growth and overweight

Repeated weight measures from birth to 6 months of age were extracted from medical records. For infants without weight measures available within ±14 days of their exact 6-month anniversary ($n = 196$, 10% of the main analysis sample), we estimated their weight at 6 months of age using preexisting sex-specific growth curves described in the literature.[32] We compared six growth models (the Count, Kouchi, first- and second-order Reed, Jenss and I-component of Karlberg models).[32] The best fit was obtained for the second-order Reed model for both sexes (data not shown). At 1 and 4 years of age, infant weight (nearest g) and length (nearest 0.1 cm) were measured by trained staff in using standard protocols. Child BMI (weight/length^2) was used to estimate age- and sex-specific *z*-scores based on the World Health Organization (WHO) referent.[33] Overweight was defined as a BMI *z*-score of $\geqslant$85th percentile. Age- and sex-specific *z*-scores for weight at birth, 6 months of age and at 1 and 4 years of age were calculated using the WHO referent.[32] In addition, we examined accelerated weight gain in infancy that has been related to a subsequent elevated risk of obesity in childhood and adulthood.[34] Rapid growth was defined as a *z*-score weight gain >0.67 s.d. between 6 months of age and birth and between 12 months of age and birth.[35] Children with a *z*-score weight gain of $\leqslant 0.67$ s.d. were characterized as slow/average growers.

Covariates

Based on previous knowledge, the following were considered *a priori* potential confounding factors: child's sex, parity, maternal age at delivery, maternal country of birth, maternal social class and education level, maternal prepregnancy BMI, maternal physical activity during pregnancy, gestational weight gain, smoking and alcohol consumption during pregnancy and maternal energy intake and fruit and vegetable consumption during pregnancy. Gestational age at birth and child's birth weight and length were considered as potential intermediate factors. Questionnaires during the first trimester of pregnancy obtained information about parity (0, 1 or more), maternal age at delivery and country of birth (Spanish vs foreign), social class (occupation during pregnancy based on the highest social class by using a widely used Spanish adaptation of the International ISCO88 coding system; I–II, managers/technicians; III, skilled; IV–V, semiskilled/unskilled),[36] education level (primary or less, secondary, university degree), prepregnancy BMI based on measured height at recruitment and prepregnancy self-reported weight (kg per square m (kg m^{-2}; (underweight (<18.5), normal weight (18.5–24.99) and overweight/obese ($\geqslant$25)). Maternal total and leisure-time physical activity in pregnancy was assessed at 12 (referred to the previous year) and 32 weeks of gestation, expressed in average metabolic equivalent scores using the procedure adapted from Norman *et al.*[37] Maternal weight throughout pregnancy was extracted from prenatal visit records and gestational weight gain was classified following the US Institute of Medicine guidelines.[38] Information on smoking (categorized as no vs yes) and alcohol consumption (defined as consumption of alcohol beverages at least 1 time/month) during pregnancy (categorized as no vs yes) was collected through questionnaires during the first and third trimesters. Maternal diet during pregnancy was assessed by a validated food frequency questionnaire;[39] fruit, vegetable and energy intake were estimated and categorized in tertiles. All questionnaires were administered face to face by trained interviewers. Information on child's sex and gestational age at birth was obtained from clinical records

Statistical analysis

Maternal plasma concentrations of 25(OH)D3 showed a seasonal distribution ($P < 0.05$, Supplementary Figure S1).To adjust for month at blood collection, we used 'deseasonalization' of 25(OH)D3 concentrations. Seasonality of 25(OH)D3 was tested by fitting the data to a sine function with a period of 12 months in a nonlinear regression cosinor model.[40,41] Then, the predicted 25(OH)D3 concentrations based on month at blood collection for each subject, derived from the sinusoidal model, were subtracted from the actual observed value. Subsequently, the overall mean was added and the resulting deseasonalized 25(OH)D3 concentrations were analyzed.[42] Maternal circulating concentration of 25(OH)D3 was evaluated as continuous (effect per 10-ng ml^{-1} decrement) and as clinically relevant categories: $\geqslant$30 ng ml^{-1} (reference group), 20–29.9 ng ml^{-1} (insufficiency) and $<$20 ng ml^{-1} (deficiency).[43]

Associations of maternal circulating 25(OH)D3 concentration with evaluated offspring phenotypes were estimated using multivariate regression analysis. All the variables significantly related to the outcomes of interest ($P < 0.20$) were included in the multivariate model, and they were retained only if they modified the coefficient of predictor variables during pregnancy by >10%. The heterogeneity of area of study-specific estimates was assessed using the *Q*-statistic. There was no evidence of heterogeneity among areas of study (Supplementary Figure S2, *Q*-statistic: $P > 0.1$), thus the results derived from pooled analyses are reported. Linear dose–response relationship between maternal circulating 25(OH)D3 concentration and offspring outcomes was assessed by using adjusted generalized additive models by graphical examination and likelihood ratio.[44] Linear relationships were observed between maternal circulating 25(OH)D3 concentration in pregnancy with assessed offspring outcomes (Supplementary Figure S3). Multivariate linear regression models were used to estimate the β-coefficients and 95% confidence intervals (CIs) for the association between maternal 25(OH)D3 concentration and continuous outcomes in offspring. For offspring prenatal growth outcomes, results are presented as percentage change in s.d. scores to enable a comparison of effect estimates between outcomes. Multivariate logistic regression models were used to estimate the association with binary outcomes; odds ratios (OR) and 95% CIs are reported.

Sensitivity analyses were performed excluding preterm deliveries and low birth weight newborns as they may follow different catch-up growth trajectories and these newborn characteristics can act as potential mediators, excluding children with estimated weight at 6 months, and after restricting the study to offspring of mothers with normal prepregnancy BMI (18.5–24.99 kg m^{-2}) in order to minimize potential effects of shared diet, lifestyle and genetic factors. Analyses were conducted by using Stata software version 12.0 (StataCorp, College Station, TX, USA) and R statistical package version 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Characteristics of the study population, overall and by area of study, are shown in Supplementary Table S1. Compared with excluded participants, mothers of those included at age 1 year had higher social class, newborns were more likely born at term and infants breastfed for longer duration, but did not differ on other characteristics (Supplementary Table S2). Median 25(OH)D3 concentration in pregnancy was 29.4 ng ml^{-1} (interquartile range, 21.9–37.2). A total of 462 (19.6%) women had vitamin D deficiency (25(OH)D3 $<$20 ng ml^{-1}) and 750 (31.8%) had insufficiency (25 (OH)D3 range 20–30 ng ml^{-1}). Table 1 shows the characteristics of mothers and offspring according to categories of maternal 25(OH) D3 concentration. Maternal age at delivery, parity, maternal social class and education, alcohol consumption in pregnancy and maternal leisure-time physical activity in week 12 of pregnancy all increased from lower to higher maternal 25(OH)D3 categories. Gestational age at blood sampling, maternal prepregnancy BMI and percentage of mothers who smoked in pregnancy all decreased from low to high categories of maternal 25(OH)D3.

Table 1. Baseline participant characteristics by maternal 25(OH)D3 concentration (ng ml^{-1}) in pregnancy

	N	*Categories of maternal 25(OH)D3 (ng ml^{-1})*			P-*value*
		< 20 (n = *462*)	*20–29.9* (n = *750*)	*⩾ 30* (n = *1146*)	
Maternal characteristics					
Season at blood sampling, %	2358				
Winter		36.8	26.0	16.9	< 0.001
Spring		35.7	26.7	21.0	
Summer		6.5	21.1	36.8	
Fall		21.0	26.3	25.2	
Gestational age (weeks) at blood sampling, mean (s.d.)	2358	13.7 (2.4)	13.6 (2.2)	13.4 (2.0)	0.020
Country of origin (foreign vs Spanish), %	2355	10.2	7.5	7.7	0.185
Maternal age (years) at delivery, mean (s.d.)	2358	30.2 (4.6)	30.4 (4.3)	31.0 (4.2)	0.001
Parity, %	2356				
0		59.9	56.8	53.8	0.163
1		34.0	37.3	38.6	
2+		6.1	5.9	7.6	
Maternal social class, %	2357				
I+II		16.0	20.5	23.6	< 0.001
III		23.2	25.5	27.9	
IV–V		60.8	54.0	48.5	
Maternal education level, %	2354				
Primary or less		28.2	23.1	24.2	0.018
Secondary		42.5	44.1	39.1	
High		29.3	32.8	36.7	
Maternal prepregnancy BMI (kg m^{-2}), %	2357				
18.5–24.99		63.6	67.6	71.7	0.024
< 18.5		4.8	5.2	4.1	
25–29.99		21.9	20.3	16.3	
⩾ 30		9.8	6.9	7.9	
Gestational weight gain, %	2276				
Recommended		36.4	38.5	38.4	0.161
Less than recommended		28.8	22.5	23.8	
More than recommended		34.8	39.0	37.8	
Maternal smoking in pregnancy, yes %	2295	24.9	17.5	16.2	< 0.001
Maternal alcohol consumption in pregnancy, yes %	2282	19.6	17.9	22.9	0.029
Total physical activity (MET) at week 12 (median, IQR)	2336	37.4 (34.9, 40.0)	37.4 (34.9, 40.2)	37.6 (34.9, 40.5)	0.529
Leisure-time physical activity (MET) at week 12 (median, IQR)	2334	2.3 (1.4, 4.3)	2.5 (1.6, 5.0)	3 (2.3, 5.0)	0.014
Total physical activity (MET) at week 32 (median, IQR)	2266	36.6 (34.4, 39.1)	36.6 (34.2, 39.5)	36.5 (34.2, 39.6)	0.985
Leisure-time physical activity (MET) at week 32 (median, IQR)	2263	3.2 (2.3, 5.0)	3.4 (2.3, 5.0)	2.5 (2.3, 5.0)	0.156
Energy intake at week 12 of gestation, %	2338				
Tertile 1		32.2	36.1	32.3	0.479
Tertile 2		33.1	31.9	34.2	
Tertile 3		34.7	32.0	33.5	
Offspring characteristics					
Child's sex male, %	2358	54.3	51.3	50.6	0.398
Preterm (< 37 weeks of gestation), %	2358	4.6	4.4	4.6	0.974
Birth weight (g), mean (s.d.)	2214	3261 (468)	3270 (469)	3266 (457)	0.949
Birth length (cm), mean (s.d.)	2187	49.5 (2.2)	49.6 (2.1)	49.6 (2.1)	0.393
Predominant breastfeeding, %	1490				
0		21.3	21.1	20.3	0.917
< 16 weeks		30.8	33.6	32.6	
16–24 weeks		35.9	34.8	34.3	
> 24 weeks		12.1	10.5	12.8	
Rapid growth 0–6months, %	1506	23.9	25.5	27.2	0.530
Rapid growth 0–12 months, %	1506	35.9	40.8	33.7	0.110
BMI for age *z*-score at age 1 year, mean (s.d.)	1498	0.34 (0.47)	0.31 (0.46)	0.26 (0.44)	0.044
Overweight at age 1 year, %	1498	33.9	31.3	26.3	0.030
BMI for age *z*-score at age 4 years, mean (s.d.)	1344	0.58 (1.1)	0.58 (1.0)	0.55 (1.1)	0.837
Overweight at age 4 years, %	1344	27.5	28.9	26.7	0.721

Abbreviations: 25(OH)D3, 25-hydroxyvitamin D3; BMI, body mass index; IQR, interquartile range; MET, metabolic equivalent.

Of the assessed offspring outcomes, BMI and overweight at age 1 year decreased across to high categories of maternal 25(OH)D. No differences were observed for maternal country of origin, gestational weight gain, maternal total physical activity and energy intake in pregnancy, child's sex, preterm delivery, birth weight and length, predominant breastfeeding, rapid growth, BMI and overweight at age 4 years.

Figure 2 shows associations of maternal 25(OH)D3 concentration in pregnancy with offspring fetal growth characteristics in each trimester of pregnancy. There was no association of maternal 25(OH)D3 concentration in pregnancy with FL and a weak inverse association with BPD at 34 weeks was observed. Lower maternal circulating 25(OH)D3 concentration in pregnancy was associated with increased AC and EFW at week 34; each 10-ng ml^{-1}

decrement in maternal 25(OH)D3 concentration in pregnancy resulted in 17% (95% CI: 2–35%) and 15% (95% CI: 0–32%) increased odds of fetal overweight defined as AC or as EFW ⩾90th percentile, respectively (Table 2). Offspring of mothers with vitamin D deficit in pregnancy showed an increased risk of fetal overweight at 34 weeks defined as AC ⩾90th percentile (OR = 1.50, 95% CI: 1.01–2.21) or as EFW ⩾90th percentile (OR = 1.47, 95% CI: 1.00–2.16; Table 2).

We did not find an association between maternal 25(OH)D3 concentration in pregnancy with offspring birth weight and length (data not shown). Maternal circulating 25(OH)D3 concentration in pregnancy was inversely associated with BMI for age *z*-score at

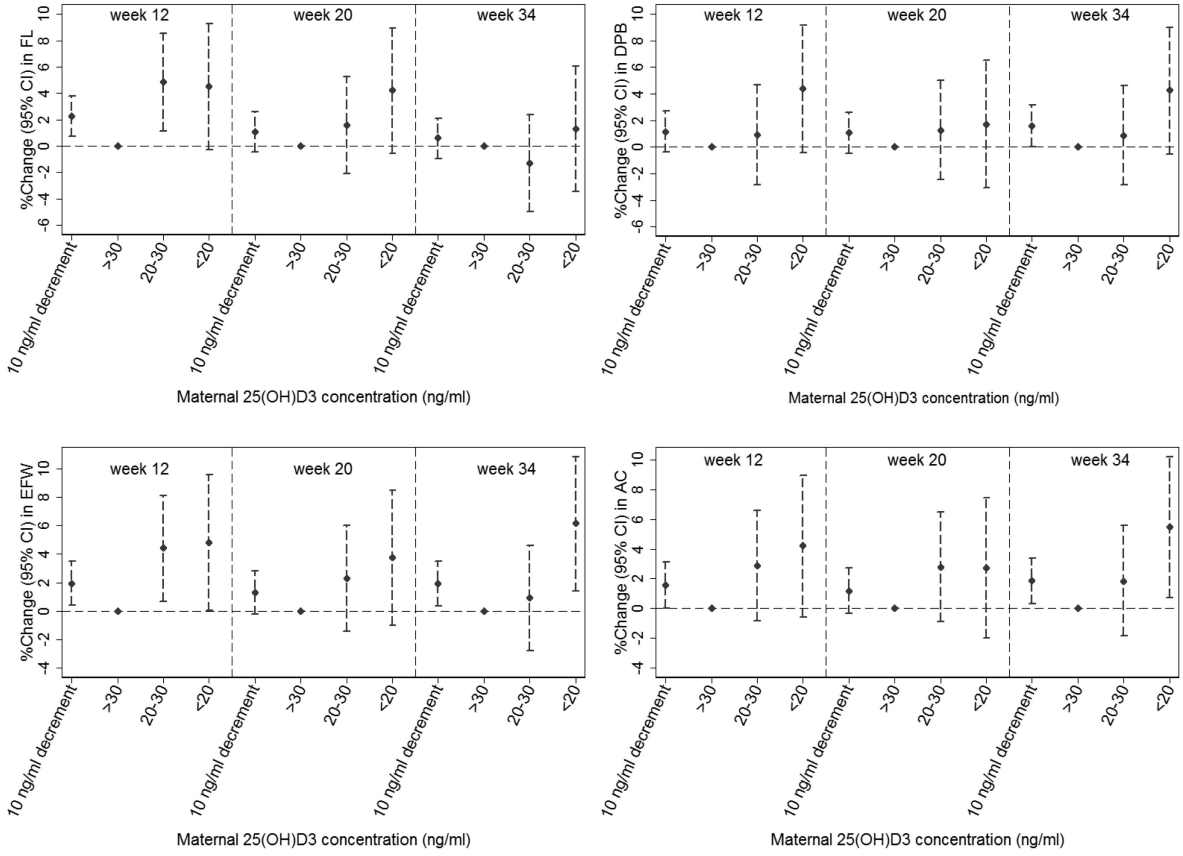

Figure 2. Associations of maternal circulating 25(OH)D3 concentration (ng ml^{-1}) in pregnancy with offspring fetal growth characteristics. Percent change in the characteristic with respect to the mean. The figure indicates deseasonalized maternal circulating 25(OH)D3 concentrations. Femur length (FL): all models adjusted for cohort, maternal social class and education, prepregnancy BMI, gestational weight gain and maternal smoking in pregnancy. Biparietal diameter (BPD): all models adjusted for cohort, maternal social class and education, parity, gestational weight gain, maternal smoking in pregnancy and NO_2 in the first trimester. Estimated fetal weight (EFW): all models adjusted for cohort, maternal social class and education, parity, prepregnancy BMI, gestational weight gain and maternal smoking in pregnancy. Abdominal circumference (AC): all models adjusted for cohort, maternal social class and education, parity, prepregnancy BMI, gestational weight gain and maternal smoking in pregnancy.

Table 2. Maternal circulating 25(OH)D3[a] concentration in pregnancy and risk of prenatal overweight[b] in offspring

	No. of cases/controls	*Per 10-ng ml^{-1} decrement*		*⩾30 ng ml^{-1}*	*20–29.9 ng ml^{-1}*		*<20 ng ml^{-1}*	
		OR (95% CI)	P-*value*		*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*
Abdominal circumference								
Week 12	226/1993	1.14 (1.00, 1.31)	0.045	1 (Ref.)	1.07 (0.78, 1.47)	0.680	1.45 (0.99, 2.11)	0.054
Week 20	212/2007	1.06 (0.93, 1.21)	0.383	1 (Ref.)	0.93 (0.67, 1.28)	0.644	1.24 (0.84, 1.82)	0.287
Week 34	226/1993	1.17 (1.02, 1.35)	0.023	1 (Ref.)	1.27 (0.92, 1.74)	0.140	1.50 (1.01, 2.21)	0.041
Estimated fetal weight								
Week 12	221/1989	1.18 (1.03, 1.36)	0.015	1 (Ref.)	1.32 (0.96, 1.81)	0.087	1.44 (0.97, 2.13)	0.068
Week 20	216/1994	1.13 (0.99, 1.30)	0.073	1 (Ref.)	1.02 (0.74, 1.41)	0.910	1.38 (0.94, 2.03)	0.097
Week 34	224/1986	1.15 (1.00, 1.32)	0.046	1 (Ref.)	1.07 (0.78, 1.48)	0.655	1.47 (1.00, 2.16)	0.046

Abbreviations: 25(OH)D3, 25-hydroxyvitamin D3; CI, confidence interval; OR, odds ratio; Ref., reference. All models adjusted for area of study, maternal social class and education, parity, maternal prepregnancy body mass index (BMI), gestational weight gain and maternal smoking in pregnancy. [a]Deseasonalized maternal 25(OH)D3 concentration. [b]Risk estimates for offspring classified ⩾90th percentile.

Table 3. Associations of maternal 25(OH)D3[a] concentration (ng ml^{-1}) in pregnancy with offspring anthropometry, rapid growth and overweight

	N	*Per 10-ng ml^{-1} decrement*		*⩾30 g ml^{-1}*	*20–29.9 ng ml^{-1}*		*< 20 ng ml^{-1}*	
		β (95% CI)	P-*value*		*β (95% CI)*	P-*value*	*β (95% CI)*	P-*value*
BMI at age 1 year	1498	0.07 (−0.001, 0.14)	0.053	0 (Ref.)	−0.001 (−0.17, 0.17)	0.983	0.18 (−0.04, 0.40)	0.107
BMI for age *z*-score at age 1 year	1498	0.04 (−0.003, 0.09)	0.066	0 (Ref.)	−0.006 (−0.12, 0.11)	0.914	0.12 (−0.03, 0.26)	0.121
BMI at age 4 years	1345	0.01 (−0.06, 0.09)	0.779	0 (Ref.)	−0.08 (−0.27, 0.10)	0.367	0.09 (−0.16, 0.33)	0.486
BMI for age *z*-score at age 4 years	1344	0.006 (−0.04, 0.06)	0.803	0 (Ref.)	−0.06 (−0.18, 0.07)	0.367	0.06 (−0.10, 0.22)	0.460
Weight *z*-score difference 0–6 months[b]	1506	−0.01 (−0.06, 0.03)	0.607	0 (Ref.)	0.04 (−0.07, 0.15)	0.437	0.02 (−0.13, 0.16)	0.834
Weight *z*-score difference 0–12 months[b]	1506	−0.02 (−0.07, 0.03)	0.399	0 (Ref.)	0.005 (−0.11, 0.12)	0.928	−0.006 (−0.16, 0.14)	0.935
	N	*Per 10-ng ml^{-1} decrement*		*⩾30 ng ml^{-1}*	*20–29.9 ng ml^{-1}*		*< 20 ng ml^{-1}*	
		OR (95% CI)	P-*value*		*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*
Overweight at age 1 year[c]	1498	1.09 (0.97, 1.22)	0.134	1 (Ref.)	1.01 (0.77, 1.32)	0.937	1.42 (1.02, 1.97)	0.039
Overweight at age 4 years[c]	1344	0.99 (0.98, 1.01)	0.404	1 (Ref.)	1.02 (0.77, 1.35)	0.876	1.19 (0.83, 1.72)	0.341
Rapid growth 0–6 months[d]	1506	1.00 (0.99, 1.01)	0.776	1 (Ref.)	1.03 (0.78, 1.34)	0.846	1.20 (0.85, 1.69)	0.304
Rapid growth 0–12 months[d]	1506	1.01 (0.99, 1.01)	0.307	1 (Ref.)	1.01 (0.79, 1.29)	0.906	0.87 (0.63, 1.20)	0.411

Abbreviations: 25(OH)D3, 25-hydroxyvitamin D3; BMI, body mass index; CI, confidence interval; OR, odds ratio; Ref., reference. All models adjusted for area of study, child's sex and age at assessment (gestational age in weeks for birth weight and length models), maternal social class and education, maternal prepregnancy BMI, gestational weight gain, maternal smoking in pregnancy and parity. [a]Deseasonalized maternal 25(OH)D3 concentration. [b]Change in s.d. scores. [c]Risk estimates for body mass index *z*-score ⩾85th percentile. [d]Risk estimates for rapid vs slow/average growers.

age 1 year (adjusted β per 10 ng ml^{-1} decrement = 0.04; 95% CI: −0.003 to 0.09; $P = 0.066$; Table 3). Moreover, maternal deficiency of vitamin D in pregnancy resulted in an up to a 42% (95% CI: 2–97%) increased odds of overweight in offspring at age 1 year. Associations between maternal circulating 25(OH)D3 concentration with offspring BMI for age *z*-score and overweight were attenuated at age 4 years (OR for overweight = 1.19, 95% CI: 0.83–1.72; Table 3). In addition, no evidence was found for an association between maternal circulating 25(OH)D3 concentration in pregnancy and offspring rapid growth from birth to ages 6 months and 1 year (Table 3).

In sensitivity analyses, estimates were essentially the same after excluding preterm deliveries, low birth weight newborns and children with estimated weight at 6 months (data not shown). Furthermore, the inverse associations of maternal circulating 25 (OH)D3 concentration in pregnancy with fetal AC, EFW and BMI and overweight at age 1 year were stronger when the analyses were restricted to offspring of women with normal prepregnancy body mass index (18.5–24.99 kg m^{-2}) (Supplementary Table S3). There was a weak association of maternal deficit of vitamin D in pregnancy with increased risk of rapid growth from birth to age 6 months (OR = 1.44, 95% CI: 0.94–2.21, $P = 0.092$) (Supplementary Table S3). However, associations of maternal circulating 25(OH)D3 concentration with rapid growth from birth to age 1 year, and offspring BMI and overweight at age 4 years remained nonsignificant.

DISCUSSION

In this large longitudinal birth cohort study, we found evidence for an association of maternal deficit of vitamin D ([that is, circulating 25(OH)D3 < 20 ng ml^{-1}) in pregnancy with increased risk of overweight in the offspring *in utero* and up to age 1 year; however, the association was attenuated at age 4 years. Moreover, the observed associations were stronger after restricting the analysis to offspring of mother with normal prepregnancy BMI. We found a weak inverse association between maternal circulating vitamin D concentrations and offspring BPD at 34 weeks of gestation, but we did not find evidence for associations for FL and rapid growth during the first year of life.

FL is commonly used as a marker of skeletal development including bone mineral content and fetal growth.[45–48] Previous studies have examined the relationship of maternal circulating 25(OH) concentration with fetal FL with inconsistent results.[3–5] We did not find evidence for an association between maternal status of vitamin D in pregnancy and offspring FL. To our knowledge, this is the first study that assesses the association of maternal vitamin D status in pregnancy and obesity-related phenotypes in offspring *in utero* using fetal biometry. We found lower maternal circulating 25(OH)D3 concentration in pregnancy to be associated with higher EFW and AC (which were highly correlated, Pearson's correlation coefficient = 0.9) and increased risk of prenatal overweight in offspring. Fetal AC is considered a good marker of higher fat depots and fetal adiposity and a predictor of the children's BMI.[31]

Previous cohort studies have examined maternal circulating 25 (OH)D concentration in pregnancy in relation to offspring BMI and fat mass during childhood.[18–20] In the current study, we found maternal deficit of vitamin D (25(OH)D3 < 20 ng ml^{-1}) to be associated with higher BMI and increased risk of overweight in offspring at age 1 year; however, associations were attenuated at age 4 years. A previous study reported deficit of vitamin D in pregnancy and greater offspring BMI at age 5 years, but not at age 9.5 years;[19] moreover, Gale *et al.*[18] did not find an association with offspring BMI at age 9 years. However, consistent inverse associations have been found between maternal circulating 25 (OH)D concentration in late pregnancy (from 28 to 34 weeks of gestation) and offspring fat mass at ages 6 and 9 years.[18–20] We did not assess offspring fat mass at either follow-up and maternal circulating 25(OH)D3 was measured in early pregnancy (around 14 weeks of gestation), complicating comparison with previous studies. None of the previous studies have examined the association of maternal circulating 25(OH)D concentration with offspring rapid growth, a good predictor of subsequent obesity.[34] We found maternal deficit of vitamin D associated with increased risk of rapid growth from birth to age 6 months in offspring of mothers with normal prepregnancy BMI, but not from birth to age 1 year, limiting these results. In addition, we did not find any evidence that birth weight and length and preterm delivery may mediate the potential associations of maternal 25(OH)D3

concentration in pregnancy and offspring growth phenotypes during infancy.

We did not find evidence that circulating 25(OH)D3 during pregnancy influenced bone growth during fetal development as reflected by the null association found with fetal FL, an indicator of linear growth. However, our results suggest that maternal circulating 25(OH)D3 concentration during pregnancy may likely influence prenatal adipose tissue development in offspring as reflected by the associations found with EFW and AC.

Shared diet, lifestyle and genetic factors could also be responsible for the association between maternal vitamin D and obesity-related phenotypes in the offspring. Although we cannot exclude this possibility, the association between maternal 25(OH)D3 concentration and obesity-related phenotypes in offspring was independent of maternal prepregnancy BMI, maternal energy intake, gestational weight gain and maternal physical activity during pregnancy. Even more, the observed associations were more evident when the analyses were restricted to offspring of mother with normal prepregnancy BMI. These results suggest that the associations we found would not be confounded by these factors.

If associations that link a poor maternal circulating 25(OH)D concentration during critical periods of development and offspring obesity-related outcomes are causal, the biological mechanisms need to be elucidated. Genetic studies have revealed diverse proteins that link vitamin D to obesity including vitamin D receptor, Toll-like receptors, the renin–angiotensin system, apolipoprotein E, vascular endothelial growth factor and poly(ADP-ribose) polymerase-1.[49] Moreover, receptors of calcitriol—the active form of the vitamin D3 metabolite—are present in adipocytes and modulate inflammatory cytokine expression.[50] In addition, vitamin D can also exert its effect on obesity through cell-signaling mechanisms, including matrix metalloproteinases, mitogen-activated protein kinase pathways, the reduced form of nicotinamide adenine dinucleotide phosphate, prostaglandins, reactive oxygen species and nitric oxide synthase.[51–54]

The present study has some limitations. Maternal circulating 25(OH)D3 concentration and anthropometric measurements were not available for all participants of the INMA Project, and this could result in selection bias especially at ages 1 and 4 years. Mothers of included participants had higher social class, and infants were breastfed for longer duration, but did not differ on other main characteristics. Although these differences may have some impact on the generalizability of results, it should not affect their internal validity. We did not measure circulating 25(OH)D2 concentrations but, normally, majority (90%) of the 25(OH)D is in D3 form. Circulating maternal 25(OH)D3 concentration was measured at only a single time point, although circulating vitamin D concentrations have been reported to remain relatively stable over long periods.[55] Direct fat mass measurements in offspring were not performed that can limit the conclusions on the role of maternal vitamin D in programming offspring adiposity. As changes in weight/BMI may reflect changes in both fat and lean mass, we cannot be sure that the associations shown here truly reflect effects of maternal vitamin D status on the development of offspring adipose tissue. Finally, lack of information on offspring circulating 25(OH)D3 concentration after birth could have resulted in some residual confounding.

The strengths of the present study include the use of a large population-based birth cohort study set up in several geographical areas of Spain and the prospective design. We evaluated a large number of potential confounders including important predictors of offspring overweight such as maternal socioeconomic status and education level, prepregnancy BMI and gestational weight gain and smoking in pregnancy. Outcomes in offspring were assessed at multiple points including fetal biometry and repeated anthropometric measurements at ages 1 and 4 years. Examination of the associations of maternal vitamin D status with offspring outcomes relied on measurement of circulating 25(OH)D3 concentrations, a reliable biomarker of vitamin D status; and we estimated mean annual circulating 25(OH)D3 concentration from a single measurement to account for seasonal variation and reduce misclassification of the exposure.

In conclusion, we found maternal deficit of vitamin D in pregnancy associated with increased risk of prenatal and early postnatal overweight and higher BMI at age 1 year in offspring; however, we found limited evidence of the association of maternal vitamin D status during pregnancy with offspring rapid growth during infancy, and BMI and overweight at age 4 years. Extended studies at older ages and examining direct obesity measures (for example, fat mass) are warranted to increase the evidence of the role of maternal vitamin D status during pregnancy in programming obesity in offspring. Given clinical and public health current concerns about vitamin D deficiency in women of reproductive age and increasing prevalence of childhood obesity, if findings are extended and replicated elsewhere, clinical trials would be warranted to determine vitamin D requirements in pregnancy and the role of vitamin D status during early-life development in programming obesity later in life.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Dr Sunyer had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design: Morales, Iñiguez, Santa Marina, Rodriguez Dehli and Sunyer. Acquisition of data: Iñiguez, Valvi, Santa Marina, Espada, Rodriguez Dehli and Sunyer. Analysis and interpretation of data: Morales, Valvi and Vrijheid. Drafting of the manuscript: Morales, Valvi and Vrijheid. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: Morales and Iñiguez. Obtained funding: Iñiguez, Santa Marina, Vrijheid and Sunyer.

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