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The association of maternal vitamin D status with infant birth outcomes, postnatal growth and adiposity in the first two years of life in a multi-ethnic Asian population: the GUSTO cohort study

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Abstract

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Conflict of Interest

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Authorship

All authors were involved in all parts of the study and approved the final manuscript. The contributions are listed below. SMS, KMG, PDG, YSC, FKPY, YSL designed and led the GUSTO study. YLO and PLQ both contributed equally to the statistical analysis and writing of the manuscript. IMA, RVD and DH provided intellectual contribution to the write-up of the manuscript. PLQ and MFFC were responsible for finalizing the manuscript. MTT conducted the data collection for the study.

Maternal vitamin D status during pregnancy has been associated with infant birth and postnatal growth outcomes, but reported findings have been inconsistent especially in relation to postnatal growth and adiposity outcomes. In a mother-offspring cohort in Singapore, maternal plasma vitamin D was measured between 26 to 28 weeks gestation, and anthropometric measurements were conducted on singleton offspring during the first 2 years of life with 3-month follow-up intervals to examine birth, growth and adiposity outcomes. Associations were analysed using multivariable linear regression. Of the total of 910 mothers, 13.2% were vitamin D deficient (< 50 nmol/L) and 26.5% were insufficient (50 to 75 nmol/L). After adjustment for potential confounders and multiple testing, no statistically significant associations were observed between maternal vitamin D status and any of the birth outcomes: small for gestational age: (OR 1.00 [95% CI 0.56- 1.79]) and pre-term birth: (OR 1.16 [95% CI 0.64- 2.11]) or growth outcomes: weight-for-age z-scores, length-for-age z-scores, circumferences of the head, abdomen and mid-arm at birth or at postnatal, and adiposity outcomes: body mass index, and skinfold thickness (triceps, biceps and subscapular) at birth or postnatal. Maternal vitamin D status in pregnancy did not influence infant birth outcomes, postnatal growth and adiposity outcomes in this cohort, perhaps due to the low prevalence (1.6% of the cohort) of severe maternal vitamin D deficiency (defined as <30.0 nmol/L) in our population.

Keywords

Maternal vitamin D; birth outcomes; growth outcomes; infant anthropometric measures; adiposity outcomes; Pregnancy

Introduction

Maternal nutrition during pregnancy is crucial for creating an optimal intra-uterine environment for fetal development(1) and has been linked to subsequent (postnatal) growth(2; 3; 4). The role of vitamin D in pregnancy is of particular interest, with reported associations between vitamin D deficiency in pregnant woman and poor offspring fetal and postnatal growth(4; 5; 6; 7).

With respect to birth outcomes, several observational studies have shown associations between maternal vitamin D deficiency (definitions in studies range from <30 to 37.5 nmol/L), or lower maternal vitamin D concentrations with higher risk of small-for-gestational-age (SGA) infants(5; 6; 8; 9; 10), pre-term births (10), and reduced birth weight, length, BMI and head circumference (5; 8; 11; 12). In contrast, studies with higher vitamin D deficiency cut-offs e.g. <50 nmol/L, >50nmol/L, <80 nmol/L (13; 14; 15; 16) or relatively high mean population level of maternal vitamin D (70 nmol/L) (17) reported lack of associations with SGA (16; 17) and birth anthropometrics measurements (13; 14; 15; 17). Taken together, results show that vitamin D supplementation during pregnancy did not affect birth weight or birth length which are in line with a Cochrane review of data from three trials (10).

Evidence on maternal vitamin D and early postnatal growth outcomes are however less clear. When defined as <30 nmol/L, maternal vitamin D deficiency was associated with greater weight-for-age in the first year of life (5; 11). Findings on length-for-age were mixed, with

maternal deficiency being associated with lower length-for-age and smaller head circumference in one study (11), but higher length-for-age in another (5). Mixed findings were also observed in studies where maternal vitamin D deficiency/insufficiency was defined at levels higher than 30 nmol/L (7; 15; 17). Thus far, current findings relating maternal vitamin D to early postnatal growth outcomes are inconclusive.

There is growing evidence to suggest the role of maternal vitamin D in infant adiposity. However, findings are also conflicting, with two studies demonstrating no association between maternal vitamin D deficiency and infant skinfold measures (13; 14) and one other study finding contradictory associations (18). Maternal vitamin D deficiency was associated with increased infant overweight risk at 1 year of age but not at 4 years (19) while in a separate study (20) lower maternal vitamin D was associated with lower fat mass in the offspring at birth but greater fat mass at ages 4 and 6 years.

In summary, the current body of work suggests that the risk of pre-term birth, SGA and smaller birth sizes are less evident when pregnant women are deficient or insufficient in vitamin D defined as >37.5 to 75 nmol/L. However, the associations of maternal vitamin D deficiency with early postnatal growth outcomes and infant adiposity are less clear, with inconsistent directionality and a smaller number of studies.

We aim to further elucidate associations of maternal vitamin D status with infant birth outcomes, early postnatal growth and adiposity outcomes up to 2 years of age in a multi-ethnic Asian mother-offspring cohort in Singapore.

Methods

Study design

Data was obtained from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study, a population-based prospective cohort study in Singapore. Further details on the GUSTO study have been previously described by Soh *et al.*(21). Pregnant women ($n=1247$) within the age range of 18-50 years were recruited from the two major public maternity units in National University Hospital (NUH) and KK Women's and Children's Hospital (KKH). Participants had to be Singaporean citizens or permanent residents, of Chinese, Malay or Indian ethnicity with parents of homogenous ethnic background, have the intention to deliver in NUH or KKH, plan to reside in Singapore in the upcoming 5 years and had to be willing to donate birth tissue at delivery (cord, placenta and cord blood). Pregnant women on chemotherapy or with pre-existing health conditions including type 1 diabetes mellitus, depression or other mental health-related disorders were excluded from the study. Written informed consent was collected from all participants upon recruitment. This study was granted ethical approval by the Institutional Review Boards of the respective hospitals involved (Clinical trial registry: NCT01174875).

Maternal and infant characteristics

Demographic data on maternal age, ethnicity, educational level, smoking status, obstetric and medical history data were collected from the women at 26-28 weeks gestation. Total maternal energy intake was ascertained from 24-hour dietary recalls conducted at 26-28

weeks gestation, and 3-day food diaries were also collected for validation purposes. Information on infant sex and birth order was obtained from birth delivery reports. Low birth weight was defined as below 2500 grams and preterm birth was defined as live birth before 37 weeks of gestation. Gestational age was determined from a dating ultrasound scan conducted in the first trimester. SGA was defined as birth weight <10th percentile for gestational age using a birth weight reference developed from 19,634 infants delivered at National University Hospital, Singapore(22).

Maternal vitamin D assessment

Maternal plasma samples collected at 26-28 weeks gestation were analyzed for serum vitamin D concentrations. The median and interquartile range of the gestational ages is presented in Table 1. Extraction of 25-hydroxyvitamin D₂ and D₃ (25(OH)D) metabolites with hexane (Chromonorm) was conducted. Plasma samples were vortex-mixed with hexane to extract 25(OH)D₂ and D₃ metabolites, after which the hexane layer was evaporated to dryness and reconstituted using methanol-water (70:30 by volume) mixture (HyperSolv). Analysis of vitamin D concentration was conducted using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Applied Biosystems) with Analyst™ software (Ver. 1.3; Applied Biosystems). Chromatographic separation was carried out with a BDS C8 reversed-phase column (ThermoHypersil). Extraction and assessment methods have been described in greater detail previously(23). The intra- and interassay CVs for 25OHD₂ and 25OHD₃ were 10.3%, and the detection limit was <4nmol/L for both metabolites.

Singapore's (1°22' N) climate which is sun-rich all year round is classified as tropical rainforest climate with no true distinct seasons, thus there should be little impact of potential seasonal variation in the 25-hydroxyvitaminD levels (Singapore Department of Statistics, 2014).

Infant anthropometric data

Measurements of infant anthropometry were adopted from standardized protocols (24). For most measurements (except skinfolds), duplicate measures are taken (skinfolds in triplicates) and averaged. Infant birth weight was obtained from birth delivery reports, and birth length was measured within 72 hours after birth using a mobile measuring mat (SECA model 210), recorded to the nearest 5 mm. Infant weight at 3, 6, 9, 12, 15, 18 months of age was measured to the nearest gram (SECA 334 Corp. Hamburg, Germany). Toddler weight at 24 months was measured to the nearest kilogram using calibrated scales (SECA 813 Corp. Hamburg, Germany). Between 3-18 months of age, recumbent infant length was measured from the top of the infant's head to the soles of the feet using an infant mat (SECA 210 mobile measuring mat), while standing height at 24 months of age was measured using a stadiometer (SECA 213 Mobile Stadiometer). Length and height (only at 24 months) measurements were taken to the scale of 0.1 cm and averaged from duplicate values.

Head circumference, abdominal circumference and mid-arm circumference measurements were similarly taken at birth, 3, 6, 9, 12, 15, 18, 24 months of age. Maximum head circumference was measured across the frontal bones of the skull and over the occipital prominence at the back of the head. Abdominal circumference was taken at the end of the

infant's normal expiration. Mid-arm circumference was measured at half the distance between the acromion process and the olecranon. All measurements were recorded using a non-stretchable measuring band (SECA 212 Measuring Tape, SECA Corp) to the nearest 0.1cm.

Skinfold measurement of the triceps and subscapular regions were taken at birth, 18 and 24 months, while measurements at the biceps were taken at 18 and 24 months. Skinfold measurement were taken in triplicates using Holtain skinfold calipers (Holtain Ltd, Crymych, UK) on the right side of the body, recorded to the nearest 0.2 mm.

Anthropometric training and standardization sessions were conducted quarterly (once every 3-months), and observers were trained to obtain anthropometric measurements that on average, were closest to the values measured by a master anthropometrist. Assessment of reliability was estimated by inter-observer technical error of measurement (TEM) and coefficient of variation (CV) (25) (Supplementary Table 1)

Statistical analysis

From 1247 mothers, 127 (10.2%) were excluded based on the following exclusion criteria: study dropouts, neonatal complications, twin birth and in vitro fertilization (IVF) birth. Twins and IVF infants were excluded due to different growth trajectories compared to singleton, naturally-conceived infants. From the remaining 1120 mothers, 910 (81.2%) had blood plasma samples available for measurement of maternal vitamin D concentrations. These mothers and their infants were included in the analysis of infant SGA and pre-term birth outcomes.

In the analysis for infant birth and postnatal growth outcomes, pre-term delivery or low birth weight (n= 103) infants were excluded, as they were expected to have different growth trajectories compared to term infants with normal birth weights. The remaining 807 subjects with complete measures were included in the analysis. Due to losses from follow-up, the number of subjects with available infant outcome measurement data at each time point varied, as reported in further detail in Figure 1.

Maternal vitamin D status was defined as deficient (<50 nmol/L), insufficient (50 nmol/L and <75 nmol/L) and sufficient (75 nmol/L) based on recommendations by Endocrine Society Clinical Practice Guideline (26). The Institute of Medicine (IOM) guidelines were not used as they define severe vitamin D deficiency as vitamin D levels <30nmol/L, of which we had very low numbers (1.6%) in our cohort to provide sufficient power for analyses. The distribution of maternal and infant characteristics between maternal vitamin D categories was compared using chi-squared test for categorical variables and ANOVA for continuous variables. Frequencies of missing demographic data were low (<5%) and hence imputed to retain good population representation. Data were assumed to be missing at random. Categorical variables were imputed as categories with the highest observed frequency in the study cohort. Continuous variables were imputed with the median values of the study cohort. Statistical significance for the descriptive data was defined as p-value<0.05.

From the anthropometric data, age and gender-adjusted z-scores for weight, length and BMI measurements were derived with reference to the WHO 2006 standards for child growth(27). Infant measures of linear growth included weight-for-age z-scores (WAZ) and length-for-age z-scores (LAZ), head, abdominal and mid-arm circumferences. Adiposity outcomes included BMI-for-age z-scores (BMIZ), and skinfold measurement from the triceps, biceps and subscapular. Conditional change in WAZ, LAZ and BMIZ across different time-points at 3-month intervals up to 24 months of age was used as a measure for gain in weight, length and BMI respectively. Z-scores at each successive time point, conditional on previous z-score, was calculated by saving the residuals from linear regression models of z-scores at each successive time point versus z-score at the earlier time point (28). Hence, the use of conditional change in z-scores between time-points is indicative of growth within the specified time period, while accounting for outcomes at earlier time points.

For binary birth outcomes of SGA and preterm birth, logistic regression analysis was used to examine the associations with maternal vitamin D status, adjusted for maternal ethnicity, education, smoking during pregnancy, age, pregnancy BMI, total maternal energy intake, infant birth order and infant gender as potential confounders. These confounders were selected if they were significantly associated with 25-hydroxyvitaminD levels or growth outcomes in univariate analysis, or reported in literature.

Separate multivariable linear regression models were used to investigate associations between maternal vitamin D status during pregnancy with infant birth outcomes, postnatal growth and adiposity outcomes. For outcome variables normalized as z-scores (WAZ, LAZ, BMIZ, conditional change in z-scores), the regression model used was adjusted for the same potential confounders in the logistic regression model but without infant gender. For other infant outcome variables (head, mid-arm and abdominal circumference, skinfold measurements), the regression model employed was adjusted for the same potential confounders in the logistic regression model.

A sensitivity analysis was conducted using a subset of infants who had growth and adiposity measurements available at all the time-points between 0 and 24 months of age (n=399). This additional analysis was conducted to test the robustness of the findings and the same multivariable linear regression models were used in the analysis.

Statistical analyses were performed on standard statistical software (SPSS Version 16.0, SPSS Inc., Chicago, IL, USA). As the postnatal growth and adiposity outcomes were taken at multiple time-points, the Bonferroni correction was used to set a statistical significance for the p-value, to account for Type I error due to multiple comparison. Statistical significance in regression models was identified by a p-value of below 0.006, determined by accounting for the maximum 8 time-points of measurement for infant outcome variables.

Results

Characteristics of participants

The maternal and infant characteristics of the study population (n=910) are summarized in Table 1. The mean maternal vitamin D concentration was 81.3 ± 27.2 nmol/L, with 13.2% of

the population who were vitamin D deficient and 26.5% who were vitamin D insufficient. Compared with mothers who were sufficient in vitamin D, deficient or insufficient mothers tended to be of Indian and Malay instead of Chinese ethnicity, were younger, had lower educational levels and had lower pregnancy BMI. The percentage of mothers who were taking vitamin D supplementation was the lowest in those who were deficient (63.9%), compared to those who were insufficient (80.6%) and sufficient (86.3%). Only 1.6% of mother's had severe vitamin D deficiency (defined as <30.0 nmol/L by the IOM guidelines). No significant differences in all the maternal or infant characteristics were observed in the cohort of $n=807$ subjects and the study subset of $n=399$ subjects (Supplementary Table 2).

Birth outcomes—Compared with mothers with sufficient vitamin D, the likelihood of having a SGA baby or a pre-term birth were not significantly different from mothers with deficient or insufficient 25-hydroxyvitamin D levels after the adjustment for potential confounders (Table 2).

Table 3, 4 and 5 show that the weight, length (WAZ and LAZ) (Table 3), head, abdominal and mid-arm circumference (Table 3), BMIZ (Table 4) and skinfold measurements from the triceps and subscapular (Table 4) at birth of infants whose mothers were sufficient in vitamin D were not significantly different to those whose mothers were insufficient or deficient in vitamin D, upon adjustment for potential confounders.

Sensitivity analysis in the subset ($n=399$) showed comparable findings, indicating no significant associations between maternal vitamin D status and infant weight and length measures, head, abdominal and mid-arm circumference, BMI and skinfold measurements at birth ($p>0.006$) (Supplementary Tables 3-6).

Postnatal growth outcomes from 3 to 24 months of age—After adjustment for potential confounding factors, the postnatal growth outcomes of weight and length (WAZ and LAZ) (Table 3), head, abdominal and mid-arm circumference (Table 3), and BMIZ (Table 4) in infants whose mothers were sufficient in vitamin D were not significantly different from infants whose mothers were insufficient or deficient in vitamin D. This was seen consistently from 3 to 24 months of age. Similarly, no associations were observed with adiposity outcomes such as and BMIZ (Table 4) and infant skinfold measurements at 18 and 24 months (Table 4).

Results from the conditional change analysis in LAZ, WAZ (Supplementary Table 7) and BMIZ (Supplementary Table 8) showed that length and weight gain in infants whose mothers were sufficient in vitamin D were not significantly different from those whose mothers were insufficient or deficient in vitamin D.

Sensitivity analysis of the subset ($n=399$) with complete postnatal growth data gave largely similar findings, such that no significant associations were observed between maternal vitamin D status and infant weight and length measures, head, abdominal and mid-arm circumference, BMI and skinfold measurements. However, we observed a non-statistically significant trend between maternal vitamin D deficiency and shorter infant length, lower weight (LAZ and WAZ), smaller BMIZ, smaller abdominal and mid-arm circumference, as

reflected by the negative beta coefficient values observed at all time-points from 3 up to 24 months of age ($p > 0.006$) (Supplementary Tables 3-5). In addition, we found a statistically significant association between maternal vitamin D insufficiency with lower infant subscapular skinfold at 24 months of age ($\beta = -0.64$, 95% CI: -1.07, -0.22; $p = 0.003$) (Supplementary Table 6).

Findings from analyses conducted using maternal plasma vitamin D concentrations as a continuous measure also did not show statistically significant associations with growth and adiposity outcomes (data not shown). Results for the unadjusted analysis are available in Supplementary Tables 9-11.

Discussion

Findings from this longitudinal cohort suggest that maternal vitamin D deficiency (25-OH-25-hydroxyvitamin D levels < 50 nmol/L) and insufficiency (50 nmol/L and < 75 nmol/L) during the mid-late trimester of pregnancy are not associated with birth outcomes, postnatal growth and adiposity outcomes in the first 2 years in a multi-ethnic Asian population.

Similar to other prospective cohort studies(16; 17) and a multi-micronutrient clinical trial(29), which used < 50 nmol/L to define vitamin D deficiency, our study showed a null association between maternal vitamin D deficiency and risk of SGA and pre-term birth. In contrast, observational studies reporting significant associations with risk of SGA and pre-term births (5; 6; 8; 9) have higher prevalence of deficiency defined by a lower vitamin D deficiency cut-off of range: 30 to 37.5 nmol/L.

Our cohort also showed null associations between maternal vitamin D status and birth measures such as birth weight and length. This observation agrees with several other cohort studies(13; 14; 15; 17), and we observe that studies reporting null findings generally defined higher cut-offs (37.5 to 80 nmol/L) for vitamin D deficiency (13; 14; 15; 17), with vitamin D prevalence's that range from (20-66%). Cohort studies(5; 11; 30) and cross-sectional studies(6; 12; 31) have reported maternal vitamin D deficiency being associated with poorer weight, length, BMI or percentile growth at birth. It was notable that these cohort studies tended to use lower vitamin D deficiency cut-offs (5; 11; 30) (range: 29.9 to 37.5 nmol/L). The cross-sectional studies however, were limited by small sample sizes(6; 12) ($N = 56$ to 70), or had used dietary data to assess maternal 25-hydroxyvitamin D levels (31).

Other anthropometric measures at birth, including infant head, abdominal and mid-arm circumference, in our study also revealed null associations with maternal vitamin D status. These findings are in contrast with two other cohort studies which found inverse relationships between maternal vitamin D concentrations and infant head circumference at birth, even though they had a high population median vitamin D concentration of 73.38 nmol/L(16) and mean vitamin D concentration of 70.5 nmol/L(17). In a study which defined a lower vitamin D deficiency cut-off (< 37.5 nmol/L), maternal vitamin D deficiency was reported to be related to lower infant head circumference at birth (30). This is however, in line with supplementation trials conducted in populations of low levels of maternal vitamin

D (mean=31.7 to 39.7 nmol/L), where most have reported the significant effects (29; 32; 33) of maternal supplementation on increasing infant head circumference. Our findings related to mid-arm circumference were supported by one study reporting similar null associations between maternal vitamin D deficiency (<30nmol/L) with mid-arm circumference at 9 months (14) while data on infant abdominal circumference are lacking.

In our study, postnatal growth outcomes were generally not affected by maternal deficiency in vitamin D during pregnancy. In the aspect of weight outcomes, our results were similar to studies by van Eijsden *et al.* and Prentice *et al.*, where no significant associations were found between maternal 25-hydroxyvitamin D levels (mean=65.9 nmol/L) with weight in children between ages 4-5 years(34), and vitamin D deficiency (<80nmol/L) with weight in infants at 2,3 and 13 week postpartum respectively(15) In contrast, a study by Eckhardt *et al.* has reported associations of maternal vitamin D deficiency (<30 nmol/L) with greater infant weight at 6 and 9 months(11). In the aspect of length outcomes, our study showed similar results as studies by van Eijsden *et al.* and Prentice *et al.*, where no associations were observed(15; 34). In contrast, Leffelaar *et al.* reported maternal vitamin D deficiency to be associated with decreased infant length at month 9 and 12(5), while Eckhardt *et al.* has found lower infant length at 1 month but higher length at 12 months(11). Lastly, in the aspect of head circumference outcomes, Prentice *et al.* again reported no significant associations with maternal vitamin D deficiency(15). However, a study by Gale *et al.* which examined postnatal growth at 9 months and subsequently at 9 years did not show any association between maternal vitamin D deficiency (<30nmol/L) and most growth outcomes (weight, length, height and mid-arm circumference), with the exception of a significant association with larger head circumference at 9 years(14). Despite the inconclusiveness of the studies surrounding postnatal growth, in general studies with lower vitamin D deficiency cut-offs below 30nmol/L found significant associations in their studies(5; 11; 14) but studies with higher cut-offs (<80nmol/L) did not(15; 34).

With regards to the associations between vitamin D deficiency and adiposity outcomes, we found no associations between maternal vitamin D with our birth and postnatal adiposity outcomes (BMI and skinfold thickness). Studies involving adiposity outcomes such as BMI are lacking and conflicting. Gale *et al.* reported no significant associations between vitamin D deficiency (deficiency defined as <30 nmol/L) with infant BMI at 9 years of age(14), while Lee *et al.* reported Vitamin D concentration(mean= 48.9-75.8nmol/L) to be inversely associated with BMI in preadolescents aged 7 to 9 years(18). In relation to skinfold thickness, Farrant *et al.*(13) reported no significant associations between maternal vitamin D deficiency during pregnancy with infant triceps and subscapular skinfold measurements at birth, which is consistent with our findings. Existing studies have not investigated maternal vitamin D with infant skinfold measurements following birth except for the preadolescent study by Lee *et al.* which has reported an inverse association between vitamin D concentrations with triceps skinfold thickness (18). Overall, there is still a lack of studies on maternal vitamin D and adiposity outcomes in early childhood which requires further investigation.

Null associations were also seen with our additional analysis of conditional z-score change in length, weight and BMI, which shows that the length gain and weight gain from 3 up to

24 months were not significantly different between vitamin D deficient mothers and those who were sufficient. None of the studies discussed have conducted similar analyses, which make comparisons difficult. However, this form of analysis provides an advantage to our study as this analytical approach enables us to examine the influence of maternal vitamin D on growth and adiposity outcomes later at time points, independent of growth and adiposity outcomes at earlier time points (35).

In the sensitivity analyses of a subset of our cohort, we do note a non-significant trend of maternal vitamin D deficiency being related to slower infant linear growth, smaller abdominal and mid-arm circumference, and lower infant BMI in the first 24 months of life. Additionally, some evidence indicating that maternal vitamin D deficiency was significantly associated with reduced infant subscapular skinfold at 24 months. This lends support to general evidence that severe maternal vitamin D deficiency can lead to poorer infant growth (5; 8; 11; 12).

The lack of significant associations in our study may be a consequence of lower prevalence of vitamin D deficiency in our population compared to cohorts in other countries. In our cohort, 13.2% were vitamin D deficient (defined as <50.0 mol/L), while only 1.6% of the cohort had severe vitamin D deficiency (defined as <30.0 nmol/L).

Strengths and limitations

To our knowledge, this is the first prospective cohort study to have comprehensively investigated the associations between maternal vitamin D during pregnancy and infant birth and growth outcomes with 3-month follow-up intervals till the age of 2 years.

The strengths of this study are the prospective study design, short intervals between follow-up sessions to track infant growth from birth and the range of outcomes that have been measured including birth outcomes, growth outcomes and adiposity outcomes. Only singleton term-born infants were included in our analysis on infant growth, while preterm and multiple birth infants were excluded due to differences in growth trajectories(36). Furthermore, the findings of our study is generalizable to other neighboring Southeast Asian countries like Indonesia (6° S) and Malaysia (2° N) which lie at the closest latitude to Singapore and which have reported vitamin D inadequacy in the pregnant women. Despite these countries receiving sunlight all year round, like Singapore, cultural practices within the different ethnic groups could contribute to the difference in the 25-hydroxyvitamin D levels. For example, the common custom is for most Malay or Muslim women to cover their skin, which could lead to lower levels of 25-hydroxyvitamin D levels. In addition, both Malay and Indian ethnic groups have darker skin pigmentation than Chinese, and would require longer duration of sunlight exposure to synthesize vitamin D₃ contributing to lower levels on vitamin D in these ethnic groups (37). In general, the better educated mothers in Singapore could also be taking steps to prevent skin cancer by using sun-protection, umbrellas or wearing clothing that protect them from the sun such as long-sleeved shirts and jeans.

Our study also has limitations that need to be addressed. Firstly, our analysis was performed only on the data of women whose vitamin D status had been measured and where growth data of the infants were available. Due to the intensive follow-up sessions, the number of

subjects decreased at later time-points, resulting in missing data at later time points, which may have affected the analytical power of our analyses.

Secondly, maternal plasma vitamin D concentration was only assessed once in late pregnancy, selected because the mobilization of vitamin D from maternal stores for fetal development is greatest during this period(4). However, assessment of plasma vitamin D concentrations at different periods of pregnancy (30) could provide useful insight into how the mobilization of maternal vitamin D stores during different pregnancy periods may affect infants differently.

Thirdly, we were unable to directly compare our results to studies that have used vitamin D cut-offs for deficiency based on the IOM guidelines (<30nmol/L). This is because only 1.6% of the mothers in our cohort have severe vitamin D deficiency (<30.0 nmol/L), which did not provide sufficient power for analyses.

Lastly, due to the observational nature of our study, there is a possibility of residual confounding arising from other factors such as infant ultraviolet-B (UV-B) exposure or infant vitamin D supplementation. The use of infant vitamin D supplementation was not taken into account in our analysis due to the low prevalence of supplementation within our cohort. Based on data from administered questionnaires at the clinic visits, only 6.92% of the infants in this study cohort were reported to have taken supplements containing vitamin D up to 12 months of age(data not shown), while 74.0% of the mothers were taking supplements containing vitamin D during pregnancy (Table 1).

A major drawback in the existing literature of vitamin D studies is the inconsistent cut-offs used to define vitamin D deficiency. In general, it was observed that studies that used lower cut-offs tended to reported significant associations between maternal vitamin D with infant outcomes(5; 8; 11), while studies that used higher cut-offs tended to report non-significant associations(13; 15; 16; 17). The inconsistency observed in past studies makes it difficult to draw reliable conclusions when these results are compared. Furthermore, and to the best of our knowledge, there are no studies looking at dose-response relationships across the vitamin D range that directly address this issue.

Conclusion

We did not find an association between maternal vitamin D deficiency and infant birth outcomes or postnatal growth and adiposity measures in our cohort. We think that this can be explained by the low prevalence of severe vitamin D deficiency within this population. Future studies employing standardized and established cut-offs defining vitamin D deficiency will enable findings across studies to be more comparable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

25(OH)D	25-hydroxyvitamin D
BMI	Body Mass Index
BMIZ	BMI-for-age z-scores
LAZ	Length-for-age z-scores
OR	Odds Ratio
SGA	Small-for-gestational-age
WAZ	Weight-for-age z-scores

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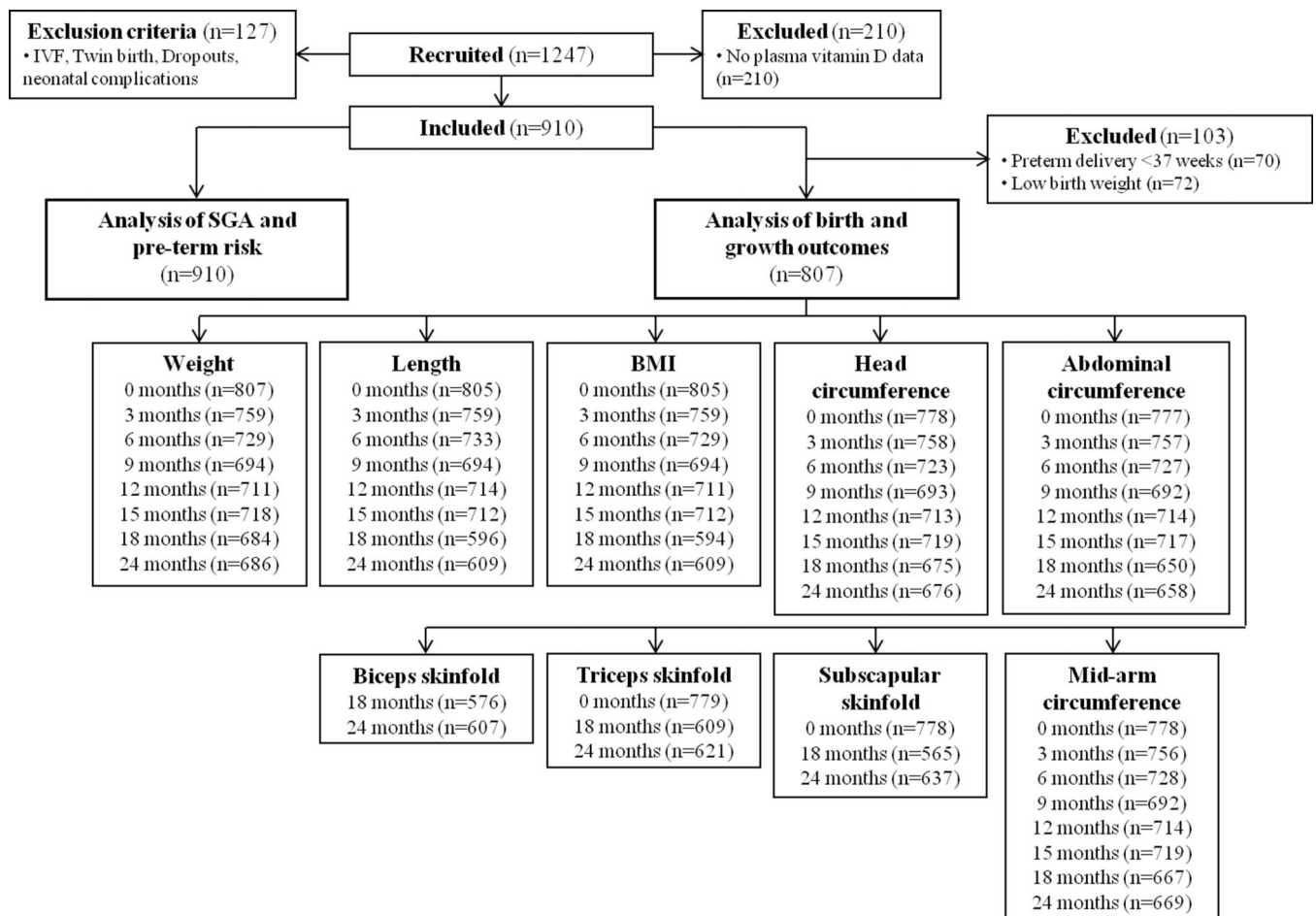


Figure 1. Flowchart of study participants with data on birth outcomes and anthropometric measurements at various time-points from birth to 24 months

Table 1

Comparison of maternal and infant characteristics of study population (n=910) between maternal vitamin D categories

	Overall <i>n</i> =910		Deficiency (<50 nmol/L) <i>n</i> =120		Insufficiency (50 and <75 nmol/L) <i>n</i> =241		Sufficiency (75 nmol/L) <i>n</i> =549		<i>p</i> -value
	Mean/ <i>N</i> ¹	SD/% ²	Mean/ <i>N</i> ¹	SD/% ²	Mean/ <i>N</i> ¹	SD/% ²	Mean/ <i>N</i> ¹	SD/% ²	
<i>Maternal characteristics</i>									
Maternal vitamin D plasma conc (nmol/L)	81.3	27.2	39.0	7.5	63.0	7.1	98.7	18.6	<0.001
Maternal age (years)	30.5	5.1	29.0	5.3	29.7	5.0	31.3	5.0	<0.001
Maternal BMI at 26 weeks³(kg/m²)	26.1	4.3	26.8	4.5	26.4	4.7	25.8	4.1	0.033
Total maternal energy intake (kcal)	2029	1288	2161	1893	1976	1180	2022	1165	0.432
Ethnicity									<0.001
Chinese	509	55.9	27	22.5	96	39.8	386	70.3	
Malay	232	25.5	56	46.7	87	36.1	89	16.2	
Indian	169	18.6	37	30.8	58	24.1	74	13.5	
Education³									0.003
Primary and Secondary	275	30.2	44	36.7	64	26.6	167	30.4	
Post-Secondary	322	35.4	42	35.0	107	44.4	173	31.5	
University	313	34.4	34	28.3	70	29.0	209	38.1	
Smoked regularly during pregnancy³									0.287
No	886	97.4	116	96.7	238	98.8	532	96.9	
Yes	24	2.6	4	3.3	3	1.2	17	3.1	
Vitamin D supplements during pregnancy⁴									0.001
No	146	16	35	36.1	41	19.4	70	13.7	
Yes	673	74	62	63.9	170	80.6	441	86.3	
Gestational age during vitamin D measurement									0.545
⁵ (Median ± IQR)	26.8	26.4-27.6	27.0	26.4-27.7	26.8	26.4-27.6	26.9	26.4-27.6	
<i>Infant characteristics</i>									
Birth weight (g)	3105.0	452.6	3143.0	414.8	3116.8	482.4	3091.5	447.2	0.474
Birth length (cm)	48.7	2.3	49.0	2.1	48.8	2.3	48.6	2.3	0.155
Birth Order									0.543
First Child	396	43.5	47	39.2	109	45.2	240	43.7	
Not First Child	514	56.5	73	60.8	132	54.8	309	56.3	
Infant sex									0.221
Female	436	47.9	56	46.7	127	52.7	253	46.1	
Male	474	52.1	64	53.3	114	47.3	296	53.9	
SGA									0.789
No	827	90.9	110	91.7	221	91.7	496	90.3	

	Overall <i>n=910</i>		Deficiency (<50 nmol/L) <i>n=120</i>		Insufficiency (50 and <75 nmol/L) <i>n=241</i>		Sufficiency (75 nmol/L) <i>n=549</i>		<i>p-value</i>
	Mean/ <i>N</i> ¹	SD/% ²	Mean/ <i>N</i> ¹	SD/% ²	Mean/ <i>N</i> ¹	SD/% ²	Mean/ <i>N</i> ¹	SD/% ²	
Yes	83	9.1	10	8.3	20	8.3	53	9.7	
Pre-term									0.919
Female	840	92.3	111	92.5	221	91.7	508	92.5	
Male	70	7.7	9	7.5	20	8.3	41	7.5	

¹ reflects mean of continuous variables or frequency for categorical variables

² reflects standard deviation of continuous variables or percentages of categorical variables

³ Missing values imputed for co-variables include: Education (12 imputed as Post-Secondary); Smoked regularly before pregnancy (3 imputed as No); Maternal BMI (16 imputed as median=25.36).

⁴ n=91 had missing data

⁵ n=8 had missing data

Table 2

Association of maternal vitamin D status in pregnancy (independent variable) with SGA and pre-term risk (dependent variables) (n=910)¹

	Vitamin D Deficiency (<50 nmol/L) <i>n=120</i>	Vitamin D Insufficiency (50 and <75 nmol/L) <i>n=241</i>	Vitamin D Sufficiency (>75 nmol/L) <i>n=549</i>
	OR (95%CI)	OR (95%CI)	OR (95%CI)
SGA ²	1.00 (0.56, 1.79)	1.08 (0.50, 2.34)	Ref
Pre-term ²	1.16 (0.64, 2.11)	1.06 (0.48, 2.38)	Ref

¹ Adjusted for maternal ethnicity, education, smoking during pregnancy, age, pregnancy BMI, total maternal energy intake, infant birth order and infant gender

² No statistically significant differences were seen between maternal vitamin D deficiency status with SGA and pre-term outcomes.

Table 3

Association of maternal vitamin D status in pregnancy (independent variable) with infant weight-for-age z-scores, length-for-age z-scores, head, abdominal and mid-arm circumferences from 0-24 months (dependent variables) (n= 807)¹

	Vitamin D Deficiency (<50 nmol/L)		Vitamin D Insufficiency (50 and <75 nmol/L)		Vitamin D Sufficiency (>75 nmol/L)	
	N	β (95%CI)	N	β (95%CI)	N	β
Weight-for-age z-score²						
0 m	109	0.09 (-0.07, 0.26)	215	0.09 (-0.03, 0.22)	483	Ref
3 m	98	-0.02 (-0.23, 0.19)	203	-0.05 (-0.21, 0.11)	458	Ref
6 m	86	0.00 (-0.23, 0.23)	195	-0.01 (-0.18, 0.16)	448	Ref
9 m	85	0.00 (-0.23, 0.23)	181	-0.01 (-0.19, 0.16)	428	Ref
12 m	89	0.01 (-0.22, 0.24)	186	0.07 (-0.10, 0.24)	436	Ref
15 m	86	-0.02 (-0.25, 0.21)	190	0.06 (-0.11, 0.23)	442	Ref
18 m	86	-0.06 (-0.30, 0.18)	184	0.11 (-0.07, 0.29)	414	Ref
24 m	79	0.00 (-0.27, 0.26)	189	0.02 (-0.16, 0.21)	418	Ref
Length-for-age z-score²						
0 m	109	0.29 (0.07, 0.51)	213	0.21 (0.04, 0.38)	483	Ref
3 m	98	-0.02 (-0.26, 0.22)	203	0.01 (-0.17, 0.19)	458	Ref
6 m	87	-0.16 (-0.43, 0.12)	196	0.05 (-0.15, 0.25)	450	Ref
9 m	85	-0.05 (-0.33, 0.22)	181	0.02 (-0.19, 0.22)	428	Ref
12 m	89	0.10 (-0.17, 0.38)	188	0.06 (-0.15, 0.26)	437	Ref
15 m	86	-0.02 (-0.29, 0.25)	189	-0.03 (-0.23, 0.17)	437	Ref
18 m	75	-0.13 (-0.41, 0.16)	164	0.21 (0.00, 0.42)	357	Ref
24 m ³	70	-0.10 (-0.39, 0.19)	168	0.04 (-0.16, 0.25)	371	Ref
Head circumference²(cm)						
0 m	103	0.06 (-0.21, 0.34)	206	0.07 (-0.14, 0.28)	469	Ref
3 m	97	-0.04 (-0.30, 0.23)	203	0.04 (-0.16, 0.24)	458	Ref
6 m	87	0.11 (-0.20, 0.41)	193	0.09 (-0.14, 0.31)	443	Ref
9 m	85	0.13 (-0.20, 0.45)	180	-0.07 (-0.32, 0.17)	428	Ref
12 m	89	0.08 (-0.24, 0.40)	188	0.00 (-0.23, 0.24)	436	Ref
15 m	86	0.10 (-0.23, 0.43)	190	-0.01 (-0.25, 0.23)	443	Ref
18 m	82	0.09 (-0.29, 0.46)	183	0.05 (-0.22, 0.33)	410	Ref
24 m	80	-0.23 (-0.59, 0.13)	186	0.03 (-0.23, 0.30)	410	Ref
Abdominal circumference²(cm)						
0 m	102	0.08 (-0.43, 0.60)	206	0.10 (-0.30, 0.49)	469	Ref
3 m	98	0.02 (-0.65, 0.68)	202	0.11 (-0.39, 0.61)	457	Ref
6 m	87	0.34 (-0.40, 1.08)	194	0.16 (-0.39, 0.70)	446	Ref
9 m	85	0.11 (-0.66, 0.87)	180	0.17 (-0.40, 0.74)	427	Ref
12 m	89	0.17 (-0.59, 0.93)	188	-0.10 (-0.66, 0.47)	437	Ref
15 m	86	0.00 (-0.74, 0.74)	188	0.12 (-0.42, 0.67)	443	Ref

	Vitamin D Deficiency (<50 nmol/L)		Vitamin D Insufficiency (50 and <75 nmol/L)		Vitamin D Sufficiency (>75 nmol/L)	
	N	β (95%CI)	N	β (95%CI)	N	β
18 m	80	-0.01 (-0.81, 0.80)	176	0.22 (-0.38, 0.81)	394	Ref
24 m	78	-0.18 (-1.06, 0.71)	178	0.17 (-0.47, 0.81)	402	Ref
Mid-arm circumference²(cm)						
0 m	102	0.04 (-0.15, 0.24)	206	-0.07 (-0.22, 0.08)	470	Ref
3 m	98	0.06 (-0.21, 0.34)	202	0.02 (-0.19, 0.22)	456	Ref
6 m	86	0.05 (-0.25, 0.34)	195	0.03 (-0.18, 0.25)	447	Ref
9 m	85	-0.02 (-0.32, 0.28)	180	-0.06 (-0.29, 0.16)	427	Ref
12 m	89	0.09 (-0.19, 0.37)	188	-0.05 (-0.25, 0.16)	437	Ref
15 m	86	0.11 (-0.19, 0.40)	190	0.02 (-0.20, 0.23)	443	Ref
18 m	83	-0.06 (-0.37, 0.25)	181	0.04 (-0.19, 0.27)	403	Ref
24 m	78	-0.04 (-0.38, 0.30)	187	-0.04 (-0.29, 0.20)	404	Ref

¹ Adjusted for maternal ethnicity, education, smoking during pregnancy, age, pregnancy BMI, total maternal energy intake, infant birth order and infant gender

² No statistically significant differences were seen between maternal vitamin D deficiency status with weight-for-age, length-for-age z-score, head circumference, abdominal circumference and mid-arm circumference.

³ No statistically significant differences were seen between maternal vitamin D deficiency status with Height-for-age –scores were measured only at 24 months

Table 4

Association of maternal vitamin D status in pregnancy (independent variable) with infant BMI-z score from 0-24 months, and infant skinfold measurements at 0, 18 and 24 months (dependent variable) (n= 807)¹

	Vitamin D Deficiency (<50 nmol/L)		Vitamin D Insufficiency (50 and <75 nmol/L)		Vitamin D Sufficiency (>75 nmol/L)	
	N	β (95%CI)	N	β (95%CI)	N	β
BMI-for-age z-score²						
0 m	109	-0.09 (-0.29, 0.12)	213	-0.02 (-0.18, 0.13)	483	Ref
3 m	98	-0.01 (-0.25, 0.22)	203	-0.07 (-0.25, 0.10)	458	Ref
6 m	86	0.11 (-0.15, 0.37)	195	-0.07 (-0.26, 0.12)	448	Ref
9 m	85	0.04 (-0.22, 0.29)	181	-0.04 (-0.23, 0.15)	428	Ref
12 m	89	-0.07 (-0.31, 0.17)	186	0.04 (-0.14, 0.22)	436	Ref
15 m	86	-0.02 (-0.27, 0.22)	189	0.09 (-0.09, 0.27)	437	Ref
18 m	75	0.01 (-0.26, 0.27)	163	0.02 (-0.18, 0.21)	356	Ref
24 m	70	0.02 (-0.27, 0.30)	168	-0.04 (-0.25, 0.16)	371	Ref
Triceps skinfold³						
0 m	103	0.12 (-0.15, 0.39)	206	-0.03 (-0.24, 0.18)	470	Ref
18 m	78	0.37 (-0.08, 0.81)	166	0.02 (-0.32, 0.35)	365	Ref
24 m	72	-0.02 (-0.52, 0.49)	171	-0.14 (-0.51, 0.22)	378	Ref
Biceps skinfold³						
18 m	76	0.29 (-0.13, 0.71)	159	-0.02 (-0.34, 0.29)	341	Ref
24 m	69	-0.05 (-0.45, 0.35)	167	-0.01 (-0.30, 0.28)	371	Ref
Subscapular skinfold³						
0 m	103	-0.07 (-0.32, 0.18)	206	0.00 (-0.19, 0.19)	469	Ref
18 m	69	0.19 (-0.18, 0.57)	155	-0.10 (-0.37, 0.18)	341	Ref
24 m	74	0.19 (-0.24, 0.61)	178	-0.30 (-0.60, 0.00)	385	Ref

¹ adjusted for maternal ethnicity, education, smoking during pregnancy, age, pregnancy BMI, total maternal energy intake and infant birth order

² No statistically significant differences were seen between maternal vitamin D deficiency status with BMI-for-age z-score.

³ No statistically significant differences were seen between maternal vitamin D deficiency status with triceps skinfold, biceps skinfold and subscapular skinfold.