The correlation between vitamin D at birth and growth at 6 months of age in twins: from a prospective longitudinal birth cohort

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Research

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Abstract

**Background:** Vitamin D deficiency is a global public health issue in women and children, is associated with adverse impacts on child growth, such as rickets. However, prior studies have mainly focused on measuring vitamin D levels in singleton pregnant women and their offspring, very limited studies have revealed the prevalence of vitamin D deficiency in twin pregnant women and their offspring. The aim of this study was to investigate vitamin D levels in twin pregnant women and their neonates. We also explored the correlation of maternal vitamin D levels with neonatal outcomes and infant growth.

**Methods:** A prospective subcohort investigation was carried out among 72 dichorionic twin pregnant mothers and their twin offspring from the Longitudinal Twin Study. Peripheral blood was collected from the mothers in the third trimester and cord blood was collected form neonates at birth to identify 25[OH]D levels. Data on the characteristics of the mothers and neonates were collected. Infant growth data and food sensitivities were also collected.

**Results:** The average maternal 25[OH]D level was 31.78 ng/mL, with 19.4% being deficiency and 20.8% insufficiency, while the average neonatal 25[OH]D level was 15.37 ng/mL, with 99.3% being deficiency or insufficiency. A positive correlation was found between maternal and neonatal 25[OH]D levels (beta-value: 0.43, 95% CI: 0.37, 0.49). Interestingly, the higher the maternal 25[OH]D level was, the smaller the co-twins birthweight discordance (beta-value: -2.67, 95% CI: -5.11, -0.23). In addition, the infants of mothers with vitamin D deficiency were more likely to be allergic to foods at six months than those of mothers with vitamin D sufficiency.

**Conclusions:** Twin neonates were at extremely high risk of vitamin D deficiency although their mothers’ vitamin D deficiency partially improved. Higher maternal vitamin D level was associated with smaller discordance of co-twins birthweight.

**Trial registration:** ChiCTR-OOC-16008203

Introduction

Vitamin D is a potent steroid hormone that is not only essential for building and maintaining bones but also plays an important role in the immune, endocrine and cardiovascular systems[1–4]. Although both animal-derived and plant-derived food sources can provide some vitamin D, the main source of vitamin D in the body depends on exposure to sunlight[5, 6]. However, the amount of time that people spend in the sun in modern society is not enough to meet their vitamin D needs[7, 8], especially since excessive ultraviolet radiation causes skin problems. In addition, ethnicity, latitude and body mass index also influence vitamin D status[9–11].

Vitamin D deficiency is a global public-health problem, especially among pregnant women and newborns[12, 13]. A deficiency of vitamin D in pregnant women increases the risk of gestational diabetes, gestational hypertension disorder and insufficient gestational weight gain and may affect fetal growth.
and bone ossification[14, 15]. Cs with a vitamin D deficiency have been shown to have a higher risk of calcemia and respiratory distress syndrome, and a higher likelihood of developing food sensitivities, asthma, type I diabetes or autism in their later life[16–20]. Previous studies have revealed that vitamin D status in the fetus and newborn is largely dependent on maternal vitamin D status, thus, the main risk factor for newborn vitamin D deficiency is maternal vitamin D deficiency[21–23]. However, the data on vitamin D status among twin pregnant women and their offspring are very limited.

In recent decades, the prevalence of twin pregnancies has increased 1.8 fold according to the National Vital Statistics Report of the US due to the development of assisted reproductive technology and advanced maternal age[24–26]. In comparison with those with singleton pregnancy, women who are pregnant with twins are considered to undergo more complex physiological changes and obviously have a higher risk of adverse obstetric consequences[27, 28]. More attention should be paid to twin pregnancies with respect to nutrition and vitamin supplementation. Thus, it is essential to clarify whether vitamin D deficiency in mothers and newborns worsens among twin pregnancies.

Therefore, in the present study, we aimed to investigate the status of 25(OH)D in mothers and their newborns in a twin pregnancies and birth cohort from Southwest China. Given the previous findings reported for singleton pregnancy, we also aimed to determine the impact of maternal 25(OH)D deficiency on maternal and neonatal outcomes as well as child growth.

**Subjects And Methods**

**Study design and participants**

The present study was embedded in the Longitudinal Twin Study (LoTiS), an ongoing twin pregnancies and birth cohort study conducted in Chongqing. Chongqing is situated in southwestern China at latitude of 29.35° N and characterized by a subtropical monsoon humid climate. This city has insufficient sunshine (1000–1400 h/year), especially in winter and spring. The LoTiS twin cohort study was established in January 2016 and aims to unravel the complex interplay between genes and the environment in specifying early life determinants of illness in infancy (Chinese Clinical Trial Registration Number:ChiCTR-OOC-16008203) and was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (No. 201530). Written informed consent was obtained from all participants. This subgroup study included dichorionic twin pregnant women with a prepregnancy BMI in the normal range (18.5 ~ 23.9) as well as daily multivitamin (vitamin D: 500 IU) supplementation from the first trimester to delivery. Mothers with chronic metabolic diseases and those using immunosuppressants were excluded. Twin pairs with a birth weight < 1500 g, significant malformations, or genetic disorders were also excluded. All twin offspring received daily usage of vitamin D supplements (400 IU/day) from birth, and had a pediatric follow-up visit thereafter at a corrected age of 6 months.

**Data Collection**

Maternal sociodemographic data (age, height, weight, education, occupation, parity, mode of conception), lifestyle behaviors before pregnancy (smoking and alcohol use) and pre-existing conditions were
collected by standardized questionnaires in the first follow-up during 11–16 gestational weeks. Information about vitamin D supplementation and other nutrients was collected using a structured questionnaire during the third trimester. Pregnancy complications, maternal and neonatal outcomes including gestational age, preterm premature rupture of membranes (PPROM, < 37 gestational weeks), neonatal gender, birthweight and small-for-gestational age (SGA, defined as a weight below the 10th percentile for GA and sex[29]) were collected from medical records.

Measurement of serum 25[OH]D and classification criteria

Peripheral blood samples were collected from the mothers in the third trimester, and cord blood samples were immediately collected from newborns after placenta delivery by using a coagulation-promoting blood collection tube. The specimen was transported to the Maternal and Fetal Medical Laboratory under refrigeration, where they were centrifuged to obtain serum. There the serum 25[OH]D level was measured by high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-MS-MS, Waters, USA), which is the gold standard method[30]. The concentrations of 25[OH]D$_3$ and 25[OH]D$_2$ were measured separately, and the total level of 25[OH]D was the sum of 25[OH]D$_3$ and 25[OH]D$_2$.

Serum 25[OH]D is the best estimator to assess the body vitamin D levels in the body[31]. For analysis, we divided mothers into 3 groups based on 25[OH]D levels: 25[OH]D levels < 20 ng/mL indicated a deficiency, 25[OH]D levels in the range of 20–30 ng/mL indicated an insufficiency, and 25[OH]D levels > 30 ng/mL were considered sufficiently high[32]. The same cut-offs were used for neonates according to the Chinese standard[33].

Infant Growth Assessment And Skin Prick Test

Infants growth was monitored for weight and length at the corrected age of 6 months. The corrected age was defined as the infant's chronological age minus the difference between term birth (40 weeks) and chronological gestational weeks of delivery. Weight and length were simultaneously measured unclothed by a digital measuring bed (Beideneng, Shanghai, China) operated by trained nurses at the Department of Child Health Care of Chongqing Health Center for Women and Children. The indexes of weight, height and BMI were converted to Z-scores for sex and age according to the WHO Child Growth Standards software (https://www.who.int/childgrowth/software/en/).

On the same day, a skin prick test (SPT) was performed to preliminarily diagnose food allergies. The SPT was performed on the volar surface of the forearm with a lancet through the use of a drop of an allergen extract and test results were produced in 15 to 20 min. The routine test screened for food types including milk, egg white, egg yolk, peanut, fish, wheat, soybean, citrus, and apple.

Statistical Methods

All statistical analyses were performed with SPSS version 25.0 (IBM, Armonk, NY, USA). Categorical variables are presented as the count and percentage, and were analyzed by the Chi-squared or Fisher's
exact test. Continuous variables are presented as means and standard deviation, and were analyzed by Student's t-test, LSD student's t test, one-way analysis of variance or the nonparametric test. Linear correlation analysis was used to explore the correlation between 25[OH]D levels in mothers and newborns and the correlation of 25[OH]D levels between co-twins. Multivariable linear regression analysis was used to detect the associations between neonatal 25[OH]D levels and related clinical characteristics. All tests were two-tailed and p < 0.05 was considered statistically significant.

Results

Subject characteristics

The selection process for this study is presented in Fig. 1. A total of 190 dichorionic twin pregnancies from LoTiS were initially recruited into this subgroup study. After excluding mother-twin offspring pairs that did not meet the inclusion criteria and did not have blood samples, a total of 72 mother-twin offspring pairs were available for the final analysis. The descriptive data of the study participants are shown in Table 1. The average maternal age at recruitment was 30.46 ± 2.93 years, and the average gestational age was 36.77 ± 1.16 weeks. The mean birth weight of all neonates was 2626.53 ± 329.96 g. In addition, 62.5% of mothers conceived with the aid of assisted reproductive technology (ART), and 66.7% of mothers delivered in the summer or autumn. The average maternal 25[OH]D level was 31.78 ± 11.1 ng/mL, with 19.5% of mothers being deficiency and 20.8% being insufficiency. Unexpectedly, the average neonatal 25[OH]D level was 15.37 ± 4.86 ng/mL, with 78.5% of neonates being deficiency and 20.8% being insufficiency.
Table 1
Description of the maternal and neonatal characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers</td>
<td>72</td>
</tr>
<tr>
<td>Age (year)</td>
<td>30.46 ± 2.93</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m2)</td>
<td>21.15 ± 1.32</td>
</tr>
<tr>
<td>Mode of conception</td>
<td></td>
</tr>
<tr>
<td>Natural conception</td>
<td>27 (37.5%)</td>
</tr>
<tr>
<td>Assisted reproductive technology</td>
<td>45 (62.5%)</td>
</tr>
<tr>
<td>Gestational weight gain (kg)</td>
<td>17.65 ± 5.89</td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td>36.77 ± 1.16</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>33 (45.8%)</td>
</tr>
<tr>
<td>Sampling season</td>
<td></td>
</tr>
<tr>
<td>Summer/autumn</td>
<td>24 (33.3%)</td>
</tr>
<tr>
<td>Winter/spring</td>
<td>48 (66.7%)</td>
</tr>
<tr>
<td>25[OH]D level (ng/mL)</td>
<td>31.78 ± 11.1</td>
</tr>
<tr>
<td>Deficiency (&lt; 20 ng/mL)</td>
<td>14 (19.5%)</td>
</tr>
<tr>
<td>Insufficiency (20–30 ng/mL)</td>
<td>15 (20.8%)</td>
</tr>
<tr>
<td>Sufficiency (&gt; 30 ng/mL)</td>
<td>43 (59.7%)</td>
</tr>
<tr>
<td>Infants</td>
<td>144</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>70 (48.6%)</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>2626.53 ± 329.96</td>
</tr>
<tr>
<td>SGA</td>
<td>5 (3.5%)</td>
</tr>
<tr>
<td>25[OH]D level (ng/mL)</td>
<td>15.37 ± 4.86</td>
</tr>
<tr>
<td>Deficiency (&lt; 20 ng/mL)</td>
<td>113 (78.5%)</td>
</tr>
<tr>
<td>Insufficiency (20–30 ng/mL)</td>
<td>30 (20.8%)</td>
</tr>
<tr>
<td>Sufficiency (&gt; 30 ng/mL)</td>
<td>1 (0.7%)</td>
</tr>
</tbody>
</table>

In the maternal vitamin D deficiency group, over a half of the mothers conceived with ART and were complicated with gestational diabetes, and 78.6% delivered in the winter or spring; this group had the
highest incidence of SGA, with the lowest birthweight as well as the highest birthweight discordance (Table 2).
Table 2
Description of the maternal and neonatal characteristics by maternal vitamin D status

<table>
<thead>
<tr>
<th>Variables</th>
<th>Deficiency</th>
<th>Insufficiency</th>
<th>Sufficiency</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers</td>
<td>N = 14</td>
<td>N = 15</td>
<td>N = 43</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>29.86 ± 3.57</td>
<td>29.87 ± 3.54</td>
<td>30.86 ± 3.52</td>
<td>0.394\textsuperscript{a}</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m\textsuperscript{2})</td>
<td>20.76 ± 1.30</td>
<td>20.50 ± 1.26</td>
<td>21.50 ± 1.62</td>
<td>0.054\textsuperscript{a}</td>
</tr>
<tr>
<td>Mode of conception</td>
<td></td>
<td></td>
<td></td>
<td>0.868\textsuperscript{b}</td>
</tr>
<tr>
<td>Natural conception</td>
<td>6 (42.9%)</td>
<td>5 (33.3%)</td>
<td>16 (37.2%)</td>
<td></td>
</tr>
<tr>
<td>ART</td>
<td>8 (57.1%)</td>
<td>10 (66.7%)</td>
<td>27 (62.8%)</td>
<td></td>
</tr>
<tr>
<td>Pregnancy weight gain (kg)</td>
<td>17.50 ± 4.97</td>
<td>19.433 ± 4.44</td>
<td>17.07 ± 6.55</td>
<td>0.412\textsuperscript{a}</td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td>36.62 ± 0.97</td>
<td>36.98 ± 1.08</td>
<td>36.73 ± 1.24</td>
<td>0.473\textsuperscript{a}</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>7 (50.0%)</td>
<td>6 (40.0%)</td>
<td>20 (46.5%)</td>
<td>0.856\textsuperscript{b}</td>
</tr>
<tr>
<td>Pregnancy-induced illness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GDM</td>
<td>8 (57.1%)</td>
<td>6 (40.0%)</td>
<td>12 (27.9%)</td>
<td>0.133\textsuperscript{b}</td>
</tr>
<tr>
<td>GHD</td>
<td>1 (7.1%)</td>
<td>1 (6.7%)</td>
<td>2 (4.7%)</td>
<td>0.510\textsuperscript{c}</td>
</tr>
<tr>
<td>ICP</td>
<td>4 (28.6%)</td>
<td>0 (0%)</td>
<td>8 (18.6%)</td>
<td>0.919\textsuperscript{c}</td>
</tr>
<tr>
<td>Sampling season</td>
<td></td>
<td></td>
<td></td>
<td>0.538\textsuperscript{b}</td>
</tr>
<tr>
<td>Summer/autumn</td>
<td>3 (21.4%)</td>
<td>6 (40.0%)</td>
<td>15 (34.9%)</td>
<td></td>
</tr>
<tr>
<td>Winter/spring</td>
<td>11 (78.6%)</td>
<td>9 (60.0%)</td>
<td>28 (65.1%)</td>
<td></td>
</tr>
<tr>
<td>Infants</td>
<td>N = 28</td>
<td>N = 30</td>
<td>N = 86</td>
<td></td>
</tr>
<tr>
<td>Gender (male)</td>
<td>12 (42.9%)</td>
<td>19 (63.3%)</td>
<td>39 (45.3%)</td>
<td>0.188\textsuperscript{b}</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>2462.50 ± 362.36</td>
<td>2736.67 ± 279.04</td>
<td>2641.51 ± 348.47</td>
<td>&lt;0.001\textsuperscript{d}</td>
</tr>
<tr>
<td>Birthweight discordance (%)</td>
<td>8.73 ± 1.13</td>
<td>6.94 ± 3.82</td>
<td>5.68 ± 4.01</td>
<td>0.048\textsuperscript{d}</td>
</tr>
<tr>
<td>SGA</td>
<td>2(7.1%)</td>
<td>1(3.3%)</td>
<td>2(2.3%)</td>
<td>0.481\textsuperscript{c}</td>
</tr>
<tr>
<td>25[OH]D level (ng/mL)</td>
<td>7.95 ± 6.05</td>
<td>12.73 ± 6.02</td>
<td>18.71 ± 5.74</td>
<td>&lt;0.001\textsuperscript{d}</td>
</tr>
</tbody>
</table>
Variables | Deficiency | Insufficiency | Sufficiency | p-value
---|---|---|---|---
BMI, body mass index; ART, assisted reproductive technology; GDM, gestational diabetes; GHD, gestational hypertension disorder; ICP, intrahepatic cholestasis of pregnancy; SGA, small for gestational age.

a Average and standard deviation. One-way Analysis of Variance
b Number (percentage). Chi-squared Test
c Number (percentage). Fisher Exact Test
d Average and standard deviation. Kruskal-Wallis Test

**Association between continuous characteristics and neonatal 25(OH)D levels**

A significant difference in neonatal 25(OH)D levels was found among the maternal vitamin D deficiency, insufficiency and sufficiency groups (Table 2). There was a directly proportional correlation between maternal 25(OH)D levels and neonatal 25(OH)D levels ($r = 0.90, p < 0.001$) (Fig. 2A). In addition, a significantly positive correlation was found between co-twins in terms of neonatal 25(OH)D level ($r = 0.91, p < 0.001$) (Fig. 2B).

Multivariable linear regression showed that neonatal 25(OH)D levels were only positively associated with maternal 25(OH)D levels (beta-value: 0.43, 95% CI: 0.37, 0.49, $p < 0.001$) and were not found to have a correlation with maternal age, BMI, gestational weight gain, gestational age, birth season or neonatal birthweight (Table 3).

Table 3

<table>
<thead>
<tr>
<th>Variables</th>
<th>Beta</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal 25(OH)D level</td>
<td>0.43</td>
<td>(0.37, 0.49)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Maternal age</td>
<td>0.05</td>
<td>(-0.13, 0.22)</td>
<td>0.589</td>
</tr>
<tr>
<td>Maternal prepregnancy BMI</td>
<td>-0.01</td>
<td>(-0.45, 0.43)</td>
<td>0.976</td>
</tr>
<tr>
<td>Maternal gestational weight gain</td>
<td>-0.02</td>
<td>(-0.14, 0.10)</td>
<td>0.725</td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>-0.56</td>
<td>(-1.22, 0.10)</td>
<td>0.093</td>
</tr>
<tr>
<td>Birth season</td>
<td>-1.04</td>
<td>(-2.39, 0.31)</td>
<td>0.129</td>
</tr>
<tr>
<td>Neonatal birth weight</td>
<td>1.45</td>
<td>(-1.05, 3.95)</td>
<td>0.252</td>
</tr>
</tbody>
</table>

**Association between maternal 25(OH)D levels and neonatal birthweight**
The neonatal birthweight and the discordance in birthweight between co-twins were significantly different between the maternal 25[OH]D deficiency and sufficiency groups (Fig. 3A-C). After adjusting for maternal age, BMI, gestational weight gain, gestational age and birth season, the results suggested that there was no correlation between maternal 25[OH]D levels and neonatal birthweight (Table 4). Surprisingly, maternal 25[OH]D levels were negatively correlated with the discordance in birthweight between co-twins, with the discordance in birthweight between co-twins decreased 2.67% when maternal 25[OH]D level increased by 1 ng/mL (95% CI: -5.11, -0.23. \( p = 0.032 \)) (Fig. 3C).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Beta</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal 25[OH]D level</td>
<td>0.01</td>
<td>(-0.01, 0.01)</td>
<td>0.513</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>0.01</td>
<td>(-0.01, 0.02)</td>
<td>0.806</td>
</tr>
<tr>
<td>Maternal prepregnancy BMI</td>
<td>0.06</td>
<td>(0.03, 0.09)</td>
<td>0.019</td>
</tr>
<tr>
<td>Maternal gestational weight gain</td>
<td>0.01</td>
<td>(0.01, 0.03)</td>
<td>0.014</td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>0.15</td>
<td>(0.11, 0.19)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Birth season</td>
<td>0.11</td>
<td>(0.02, 0.20)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Impact of maternal 25[OH]D level on infant growth and food sensitivities

At six months of corrected age, the z-scores of the weight-for-age, height-for-age, weight-for-height, and BMI-for-age indexes showed no differences among the maternal 25[OH]D deficiency, insufficiency and sufficiency groups for all infants taking vitamin D supplements each day. However, in the maternal vitamin D deficiency group, the incidence of allergies to foods was highest (Table 5).
### Table 5
Description of the growth and food sensitivity by maternal 25[OH]D status

<table>
<thead>
<tr>
<th>Variables</th>
<th>Deficiency (N = 28)</th>
<th>Insufficiency (N = 30)</th>
<th>Sufficiency (N = 86)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHZ</td>
<td>0.39 ± 0.80</td>
<td>0.47 ± 0.73</td>
<td>0.35 ± 0.82</td>
<td>0.828a</td>
</tr>
<tr>
<td>WAZ</td>
<td>0.41 ± 0.88</td>
<td>0.59 ± 0.97</td>
<td>0.34 ± 0.85</td>
<td>0.527a</td>
</tr>
<tr>
<td>HAZ</td>
<td>0.29 ± 1.10</td>
<td>0.57 ± 1.21</td>
<td>0.42 ± 0.95</td>
<td>0.673a</td>
</tr>
<tr>
<td>BAZ</td>
<td>0.34 ± 0.66</td>
<td>0.38 ± 0.98</td>
<td>0.36 ± 0.79</td>
<td>0.981a</td>
</tr>
<tr>
<td>Allergic to one or more food</td>
<td>4 (14.3%)</td>
<td>2 (6.7%)</td>
<td>4 (4.7%)</td>
<td>0.200b</td>
</tr>
</tbody>
</table>

WHZ, z-score for weight-for-height; WAZ, z-score for weight-for-age; HAZ, z-score for height-for-age; BAZ, z-score for BMI-for-age.

*a* Average and standard deviation. Student t test

*b* Number (percentage). chi-square test

### Discussion

In this prospective preliminary study, we reported that 19.5% of mothers being vitamin D deficiency, and their neonates had a remarkably high prevalence of vitamin D deficiency at birth, with a rate of 78.5%. We noticed very poor vitamin D stores especially in twin neonates, even though all mothers took multivitamins (including vitamin D: 500 IU) daily during pregnancy.

Several studies conducted on singleton pregnancy among the Chinese population have investigated maternal 25[OH]D levels. Maternal vitamin D deficiency was reported in 79.2% of a multiethnic population without an investigation of prenatal vitamin D supplementation[34]. Approximately 10% of mothers took prenatal vitamin D, and the maternal vitamin D deficiency rate was also high at 74.9%. If the mothers took prenatal vitamin D daily during the last one month before delivery or over three times per week, the maternal vitamin D deficiency rates improved to 36.6% and 31.6%, respectively[35]. The maternal vitamin D deficiency rate in this study was lower than that in the aforementioned studies. This can be explained by the fact that all subjects in this study took vitamin D supplements daily starting in the first trimester. Thus, we speculated that a high frequency of vitamin D supplementation during pregnancy is an effective way to reduce the risk of maternal vitamin D deficiency.

Previous studies have revealed that the rates of maternal and their neonatal vitamin D deficiency are comparable in singleton pregnancy[34, 36]. A similar statement was also found in the northern Indian twin pregnancies population, with a maternal vitamin D deficiency rate of 90% and neonatal vitamin D
deficiency rate of 89%[13]. However, the results of our study showed that the neonatal vitamin D deficiency rate was 4-fold higher than the maternal vitamin D deficiency rate, which was inconsistent with the aforementioned study. This is probably attributed to the distinct rates of maternal vitamin D deficiency in the two studies. The significant difference in neonatal vitamin D deficiency and maternal vitamin D deficiency rates in our study was likely due to the maternal vitamin D supply to the two fetuses. In addition, we also found that the maternal vitamin D level was an independent factor that correlated with the neonatal vitamin D level, which was consistent with previous studies[37].

The clinical practice guidelines of the Endocrinology Society recommend that pregnant women should take vitamin D supplements of at least 600 IU daily. However, twin neonates were at a very high risk of vitamin D deficiency when twin pregnant women took vitamin D supplements of 500 IU daily. Therefore, further investigations are needed to establish an appropriate dose that is effective for improving neonatal vitamin D deficiency and safe for the mothers’ metabolism.

There are conflicting reports about the association between maternal vitamin D levels and neonatal birthweight in singleton pregnancy. Hossein Hajianfar et al found a significant inverse association between maternal vitamin D level and the rate of low birthweight neonates[38]; others have reported no relationship[39, 40]. In our study, although the neonatal birthweight in the maternal vitamin D deficiency group was lower than that in the other group, no correlation was found between maternal vitamin D level and neonatal birthweight after adjusting for potential confounders. Interestingly, we found that the higher the maternal vitamin D level was, the smaller the discordance in birthweight between co-twins, thus presenting a negative association. However, further investigations are needed to detect the relative molecular mechanism.

Vitamin D deficiency in neonates has been shown to lead to a higher risk of food sensitivities in later life. In our study, because 99.3% of neonates were vitamin D deficient or insufficient, we compared the status of infants allergic to foods by maternal vitamin D levels. Although no significant difference was found among the maternal vitamin D deficiency, insufficiency and sufficiency groups in terms of allergies to foods, the allergy rate was highest in the maternal vitamin D deficiency group. It is worth exploring whether maternal vitamin D deficiency or neonatal vitamin D deficiency predominantly influences infant allergies to foods.

The strength of our study is the specialized study population. We used strict inclusion and exclusion criteria to screen the participants. Monochorionic twin pregnant women were not selected due to the higher risks of maternal and fetal complications, higher rate of preterm birth and lower neonatal birthweight. Prepregnancy BMI also has an obvious impact on perinatal outcomes, so we only recruited women with prepregnancy BMIs in the normal range. Additionally, the food sensitivities of infants at six months were followed, representing a relatively complete study design.

This preliminary study contributes new knowledge about the status of maternal and neonatal vitamin D levels in twin pregnancies, but several limitations of this study should be taken into consideration. First, compared with that of similar studies in singleton pregnancy, the sample size in this study is relatively
small. Second, pregnant women in our hospital were routinely advised to take multivitamin supplements daily based on the clinical guidelines, particularly twin pregnant women; thus, there were no women who did not take multivitamin supplements as controls. Finally, singleton pregnant women taking the same multivitamin supplements and their neonates should be included as a control group. A large-scale study including singleton pregnancy and twin pregnancies and conducted in multiple centers is essential to better understand the prevalence of maternal and neonatal vitamin D deficiency in China among twin pregnancies populations.

Conclusions

In conclusion, this study suggested that despite of twin pregnant women taking prenatal vitamin D supplements and the mothers’ vitamin D deficiency partially improving, their twin neonates were at extremely high risk of vitamin D deficiency. These findings indicated that the obstetrician should give special attention to the dose of vitamin supplements provided to the twin pregnancy population.

Abbreviations

NC: Natural conception group; IVF: in vitro fertilization; BMI: Body mass index; CI: Confidence interval; GA: Gestational age; GDM: Gestational diabetes mellitus; ICP: Intrahepatic cholestasis of pregnancy; LoTiS: Longitudinal Twin Study; OR: Odds ratio; PE: Preeclampsia; SGA: small for gestational age; ART: assisted reproductive technology; GHD: gestational hypertension disorder; WHZ: z-score for weight-for-height; WAZ: z-score for weight-for-age; HAZ: z-score for height-for-age; BAZ: z-score for BMI-for-age.

Declarations

Ethics approval and consent to participate

The experimental design and procedures were approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (No. 201530).

Consent for publication

Not applicable.

Availability of data and materials

All of original data are available upon reasonable request made to the corresponding author.

Competing interests

The authors declare that they have no competing interests.
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**Author contributions:** CT and LW designed the research protocol; XL and JY conducted the study; XL analyzed the data; XL drafted the manuscript; WL, CT, RS, MK, PB critically revised the manuscript; CT, QT, HQ provided funding resource. All authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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**References**


**Figures**
Dichorionic twin pregnancies from LoTiS
n=190

- 3 women chronic metabolic diseases
- 44 women with BMI ≥ 24.0
- 21 women with BMI < 18.5
- 5 women without routine usage of vitamin D

Eligible dichorionic twin pregnancies
n=117

- 25 either mother or neonates without blood sample
- 3 either twin neonates with birth weight <1500 g
- 17 twin intants without postnatal follow-up

Analytic sample
n=72

Figure 1

The selection process for this study.

Figure 2

A

B

Maternal 25(OH)D levels (ng/mL)

Neonatal 25(OH)D levels (ng/mL)

Lighter co-twin 25(OH)D levels (ng/mL)

Heavier co-twin 25(OH)D levels (ng/mL)

R=0.90
P<0.001

R=0.91
P<0.001
Correlation between maternal and neonatal 25\(\text{OH}\)D levels and correlation of co-twins’ 25\(\text{OH}\)D levels. A) There was a directly proportional correlation between maternal 25\(\text{OH}\)D levels and neonatal 25\(\text{OH}\)D levels \((r=0.90, p<0.001)\); B) A significantly positive correlation was found between co-twins in terms of neonatal 25\(\text{OH}\)D level \((r=0.91, p<0.001)\).

Figure 3

The impact of maternal 25\(\text{OH}\)D levels on neonatal birthweight. A) The neonatal birthweight was significantly different between the maternal 25\(\text{OH}\)D status (deficiency group vs insufficiency group, \(P<0.001\); deficiency group vs sufficiency group, \(P=0.011\)); B) The neonatal birthweight discordance(%) was significantly different between the maternal 25\(\text{OH}\)D status (deficiency group vs sufficiency group, \(P=0.016\)); C) Maternal 25\(\text{OH}\)D levels were negatively correlated with the discordance in birthweight between co-twins, with the discordance in birthweight between co-twins decreased 2.67% when maternal 25\(\text{OH}\)D level increased by 1 ng/mL (95% CI: -5.11, -0.23. \(p=0.032\)).