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# Maternal periconception serum vitamin B12 concentration and risk of preterm birth: a prospective cohort study

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## ABSTRACT

**Background** Maternal vitamin B12 levels during pregnancy have been implicated in the risk of preterm birth, but current evidence remains controversial and just focuses on measurements during pregnancy.

**Objective** To assess the association of maternal periconception serum vitamin B12 concentration, including levels before conception and at early gestation, with the risk of preterm birth and its subtypes in a large Chinese population.

**Design** Prospective cohort study.

**Participants** A total of 26 977 women from Shanghai, China, with serum vitamin B12 concentration measured either before conception or at early gestation.

**Outcome measures** Preterm birth was defined as delivery before 37 weeks of gestation and was stratified as preterm birth <32 and <34 weeks. Cox regression was used to estimate the association between maternal vitamin B12 and preterm birth.

**Results** Overall, 1599 (5.9%) of participants delivered preterm. The median periconception vitamin B12 concentration was 483.0 (IQR, 368.0–622.0) pg/mL. No evidence was observed of an association between maternal vitamin B12 concentration and risk of preterm birth (per 100 pg/mL increment: adjusted hazard ratio (aHR), 0.99; 95% CI, 0.96 to 1.02, *P*=0.572). Similarly, null associations were observed for preterm birth subtypes (aHR, 0.98 [0.89 to 1.09] and 0.97 [0.90 to 1.05] for preterm birth <32 and <34 weeks, respectively).

**Conclusions** No evidence of associations was found between maternal vitamin B12 concentration and risk of preterm birth in a population with relatively sufficient vitamin B12 levels. Future studies in populations with varied baseline levels of vitamin B12 are needed to validate these findings across different populations and regions.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the first large prospective cohort study in the Chinese population quantifying the association of maternal periconception serum vitamin B12 with preterm birth and its subtypes.
⇒ The study uniquely investigated vitamin B12 concentrations before conception.
⇒ Generalisability is limited by the relatively sufficient vitamin B12 levels compared with other populations.
⇒ Residual confounding cannot be completely ruled out.

## INTRODUCTION

Preterm birth, defined as delivery before 37 weeks of gestation,[1] affects 4%–16% of pregnancies and results in approximately 13.4 million preterm infants annually.[2] Roughly 5% of preterm births occur before 28 weeks, 10% between 28 and 32 weeks, 10%–20% between 32 and 34 weeks and 60%–70% between 34 and 36 weeks.[3 4] Complications of preterm birth remain the leading cause of mortality among children under 5,[5 6] particularly for infants born before 32 weeks.[7 8] Survivors of very or extremely preterm birth frequently face lifelong challenges, including disability, neurodevelopmental disorders[9 10] and increased long-term risks of cardiovascular and metabolic diseases.[11 12]

Preterm birth is considered a multifactorial syndrome,[3] in which maternal periconception nutritional status is a key modifiable risk factor. Vitamin B12 (cobalamin) is an essential water-soluble micronutrient involved in one-carbon metabolism and functions as a coenzyme in folate and methionine cycles, processes integral to DNA methylation and cellular metabolism.[13 14] Previous studies have demonstrated that vitamin B12 is crucial for early development and perinatal health,[15 16] with increased requirements during pregnancy owing to foetal deposition and enhanced absorption.[17]

However, current evidence linking maternal vitamin B12 concentrations with preterm birth is inconsistent. A meta-analysis reported a linear association between maternal vitamin B12 deficiency (<148 pmol/L) during pregnancy and a 1.21-fold increased risk of preterm birth.[18] In contrast, a Singaporean cohort study found no significant association for maternal vitamin B12 during 26–28 weeks of gestation,[19] and a small cross-sectional

study in India even noted higher vitamin B12 levels before delivery among preterm cases.[20]

In addition to population differences and methodological limitations, whether and to what extent maternal serum vitamin B12 is associated with preterm birth in Chinese women remains unclear. Moreover, the relationship between maternal periconception vitamin B12 levels—particularly those measured before conception—and preterm birth risk has not been reported to date.

Therefore, this study aimed to evaluate the association between maternal periconception serum vitamin B12 concentration, including levels before conception, and the risk of overall and subtype-specific preterm birth using data from a large prospective cohort. Our findings provide population-based evidence to inform early prevention strategies for preterm birth through vitamin supplementation.

## METHODS

### Study design and participants

This prospective cohort study is based on the Shanghai Preconception Cohort (SPCC; NCT02737644), which is an ongoing cohort designed to investigate the associations between periconception essential nutrients and maternal and neonatal outcomes. Detailed information on the SPCC design has been reported previously.[21 22] Briefly, couples attending preconception clinics (intending to conceive within 1 year) or pregnant women undergoing their first prenatal examination (within 14 weeks of gestation) were recruited. Demographic, anthropometric and lifestyle data were collected via a standardised questionnaire. All pregnant participants were followed up with routine prenatal healthcare, and obstetric data were obtained from electronic medical records. Delivery outcomes were available for 28 254 women until October 2023. Women missing gestational age data, with gestations exceeding 43 weeks or with invalid blood samples, were excluded.

The study was approved by the Institutional Review Board at the Children's Hospital of Fudan University (IRB number 201649), and all participants provided written informed consent. This reporting of the study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.

### Serum vitamin B12 concentration measurement

Fasting blood samples were collected from the peripheral veins at recruitment, either before conception or at early gestation, in accordance with standard protocols described elsewhere.[21] Serum vitamin B12 concentrations were measured using a chemiluminescence microparticle immunoassay (ARCHITECT i2000SR Analyzer, Abbott Laboratories, IL, USA) with daily quality control using three standard solutions. The inter-assay coefficient of variation was <7.5%, and the intra-assay coefficient was <6.5%.

### Definition of preterm birth

Gestational age was estimated from the first day of the last menstrual period and confirmed by ultrasonography. Preterm birth was defined as gestational age at delivery <37 weeks according to the international guideline[1] and can be further categorised as extremely preterm (<28 weeks), very preterm (28 to <32 weeks), moderate preterm (32 to <34 weeks) and late preterm (34 to <37 weeks).[3] To accommodate the time-to-event analyses, preterm birth <32 and <34 weeks of gestational age were treated as two subtypes of preterm birth.

### Covariates

Maternal demographic information included age, ethnicity, educational attainment, parity and body mass index (BMI). Preconception BMI was calculated as weight (kg) divided by the square of height (m) according to the self-reported questionnaire. Smoking exposure was defined as cigarette smoking or exposure to secondhand smoke within 3 months before conception or during pregnancy, and drinking was similarly defined. Multivitamin supplementation referred to the intake of supplements containing multivitamins before or after conception. Pregnancy complications, including gestational diabetes mellitus (GDM) and hypertensive disorders of pregnancy (HDP), were obtained from medical records.

### Statistical analysis

Participant characteristics were summarised using appropriate descriptive statistics, and differences between preterm and term birth groups were compared.

Cox regression was used to assess the associations of maternal vitamin B12 concentration with the risk of preterm birth, with the gestational age at delivery as the time scale. This approach allows for the assessment of variations in the risk of preterm birth with gestational age.[23] The proportional hazard test and Schoenfeld residuals were used to evaluate the proportional hazard assumption, and all Cox models met the assumptions. The hazard ratio (HR) and 95% confidence interval (CI) for preterm birth were reported. Vitamin B12 concentration was treated as a continuous variable (per 100 pg/mL increment). To aid in clinical interpretation and comparison with previous studies, vitamin B12 concentration was categorised as <200, 200–300 and ≥300 pg/mL (equivalent to <148, 148–221 and ≥221 pmol/L, respectively) according to the commonly used clinical thresholds for vitamin B12 deficiency and marginal deficiency.[24 25] Given the very low prevalence of vitamin B12 deficiency in our study population, deficiency and marginal deficiency (<300 pg/mL) were combined, with ≥300 pg/mL as the reference. The directed acyclic graph was used to identify the variables that might confound the association between vitamin B12 and preterm birth (online supplemental figure 1). Model 1 was a simple model with only adjustment for the duration between enrolment and conception (calculated as the enrolled date minus the first date of the last menstrual period, in months) to minimise the influence of different

blood-drawing time windows. Model 2 was additionally adjusted for maternal age, preconception BMI, parity, smoking exposure, drinking, multivitamin supplementation, GDM and HDP and was treated as the main analysis.

Cox models were repeated for preterm birth subtypes. To assess potential non-linear relationships, restricted cubic splines (RCS) were employed. The RCS with three knots (10th, 50th and 90th percentiles) were selected to balance flexibility and overfitting (using Akaike's information criterion) according to Harrell's recommendation.[26] Subgroup analyses were conducted for participants enrolled before conception vs early gestation, and first-trimester vitamin B12 was further classified using thresholds of <122 pg/mL for deficiency and <252 pg/mL for impaired intracellular status that were reported in a previous study.[27] Additionally, in women with both preconception and early gestation measurements, longitudinal changes were analysed in relation to preterm birth risk. Missing data were imputed using multiple imputation by chained equations (10 datasets, R package "mice"), and sensitivity analyses were performed among women with complete vitamin B12 measurements. To account for districts clustering, mixed-effect models with recruitment districts as the random effect were additionally applied to assess the robustness of the main results.

In this study, the standard deviation (SD) for vitamin B12 (per 100 pg/mL increment) was 2.15, with approximately 5% of the variance explained by covariates. Given a two-sided alpha of 0.05 and a 6% event rate for preterm birth, our current sample size (n=26 977) provided 80% power to detect an HR≤0.97 or HR≥1.03 using Cox regression (online supplemental table 1). Power calculations were performed using PASS software (version 15.0).

All statistical analyses were performed in R (version 4.3.1), with two-sided $P<0.05$ considered statistically significant. No adjustments were made for multiplicity.

### Patient and public involvement

No patients or members of the public were involved in the design or implementation of this study.

## RESULTS

### Participant characteristics

Among 26 977 women, 14 472 were enrolled before conception and 12 505 at early gestation. A total of 1 599 women (5.9%) delivered preterm, including 170 before 32 weeks and 339 before 34 weeks (figure 1 and table 1). The mean age of the participants was 30.7 ± 3.7 years, with an average preconception BMI of 21.2 ± 2.8 kg/m$^2$. The cohort was 98% Han, 16.7% attained postgraduate education and 94.3% were primiparous. Detailed characteristics are presented in table 1. Maternal characteristics were similar between women with and without vitamin B12 measurement (online supplemental table 2). Compared with term birth, women who delivered preterm had a higher prevalence of age >35, lower educational attainment, overweight/obese (BMI≥24 kg/m$^2$), primiparity, smoking exposure, GDM and HDP (online supplemental table 3). Similar patterns were observed for preterm subtypes (online supplemental table 4).

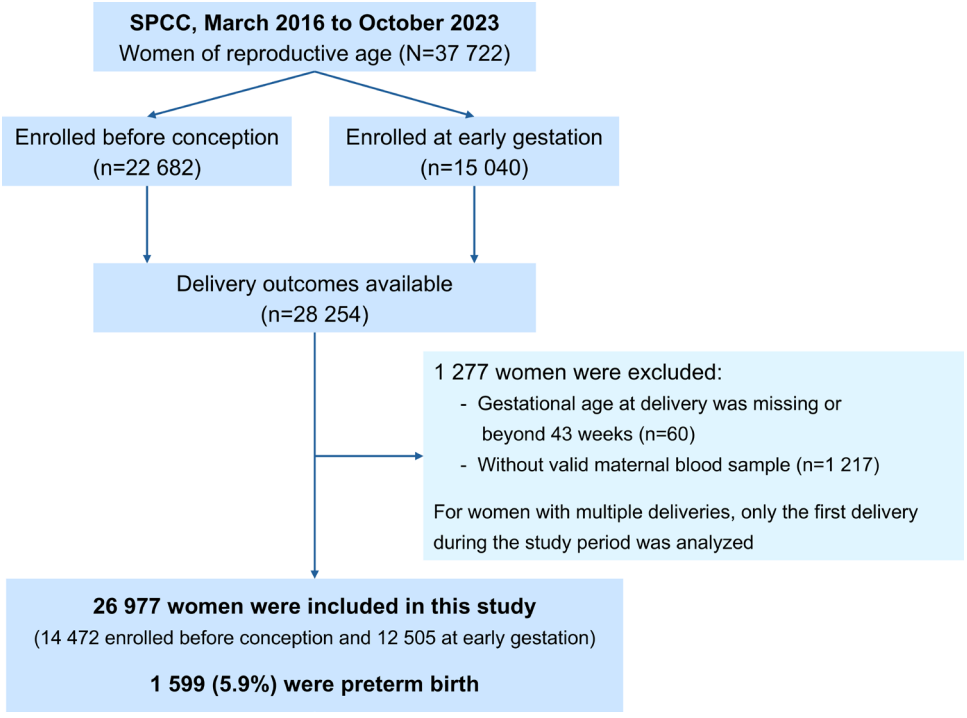


**Figure 1** Flowchart of the study population. A total of 26 977 women of reproductive age were included in this study, of which 1599 (5.9%) were preterm births. Abbreviation: SPCC, Shanghai Preconception Cohort.

Table 1 Characteristics of the study participants

| Characteristics | Overall (n=26 977) | Enrolled before conception (n=14 472) | Enrolled at early gestation (n=12 505) |
|---|---|---|---|
| **Maternal characteristics** | | | |
| Age, year, mean (SD) * | 30.7 (3.7) | 30.5 (3.5) | 30.9 (3.9) |
| Han ethnicity, n (%) * | 25 546 (98.0) | 13 643 (98.2) | 11 903 (97.8) |
| Educational attainment, n (%) * | | | |
| High school | 2479 (9.4) | 849 (6.0) | 1630 (13.4) |
| College | 19 565 (74.0) | 10 828 (75.9) | 8737 (71.7) |
| Postgraduate | 4412 (16.7) | 2591 (18.2) | 1821 (14.9) |
| Preconception BMI, kg/m$^2$, mean (SD) * | 21.2 (2.8) | 21.0 (2.7) | 21.3 (3.0) |
| Parity, n (%) | | | |
| Primipara | 25 448 (94.3) | 14 136 (97.7) | 11 312 (90.5) |
| Multipara | 1529 (5.7) | 336 (2.3) | 1193 (9.5) |
| Smoking exposure, n (%) * | 4523 (19.0) | 2849 (21.2) | 1674 (16.3) |
| Drinking, n(%) * | 5325 (22.4) | 4094 (30.8) | 1231 (11.8) |
| Multivitamin supplementation, n(%) * | 9499 (56.4) | 2521 (45.2) | 6978 (62.0) |
| GDM, n (%) | 3637 (13.5) | 468 (3.2) | 3169 (25.3) |
| HDP, n (%) | 721 (2.7) | 325 (2.2) | 396 (3.2) |
| Duration between enrolmentt and conception, month, median (P25, P75) † | −1.6 (-10.8,2.5) | −8.3 (-18.4,−3.1) | 2.7 (2.2,2.9) |
| **Maternal serum vitamin concentration** | | | |
| Serum vitamin B12, pg/mL, median (P25, P75)* ‡ | 483.0 (368.0,622.0) | 499.0 (382.0,643.0) | 465.0 (353.0,598.0) |
| Deficiency (<200 pg/mL), n (%) | 608 (2.4) | 265 (1.9) | 343 (2.9) |
| Marginal deficiency (200–300 pg/mL), n (%) | 2555 (10.0) | 1157 (8.5) | 1398 (11.8) |
| Sufficiency (≥300 pg/mL), n (%) | 22 300 (87.6) | 12 221 (89.6) | 10 079 (85.3) |
| **Spectrum of preterm birth** | | | |
| Preterm birth, n (%) | 1599 (5.9) | 844 (5.8) | 755 (6.0) |
| Preterm birth <32 weeks, n (%) | 170 (0.6) | 75 (0.5) | 95 (0.8) |
| Preterm birth <34 weeks, n (%) | 339 (1.3) | 167 (1.2) | 172 (1.4) |

*Data were missing for 0.07% (n=19) in age, 3.4% (n=916) in ethnicity, 1.9% (n=521) in educational attainment, 0.7% (n=193) in preconception BMI, 12.0% (n=3229) in smoking exposure, 12.1% (n=3252) in drinking, 37.5% (n=10 137) in multivitamin supplementation and 5.6% (n=1514) in serum vitamin B12 concentration.
†The duration between enrollment and conception was calculated as the enrolled date minus the date of the last menstrual period, with preconception accruals as negative numbers and post-conception accruals as positive numbers. 5.4% (n=1446) of data on duration between enrollment and conception were missing.
‡To convert the vitamin B12 concentration from pg/mL to pmol/L, multiply by 0.7378.
BMI, body mass index; GDM, gestational diabetes mellitus; HDP, hypertensive disorders of pregnancy.

### Serum vitamin B12 concentration

The overall median serum vitamin B12 concentration was 483.0 (IQR, 368.0–622.0) pg/mL, with 499.0 (382.0–643.0) pg/mL among women enrolled before conception and 465.0 (353.0–598.0) pg/mL among those enrolled at early gestation, respectively. Vitamin B12 deficiency and marginal deficiency were present in 2.4% and 10.0% of the participants, respectively (table 1). Distributions of vitamin B12 were similar across enrolment periods (online supplemental figure 2). Women who delivered preterm had marginally lower vitamin B12 levels than those with term births (472.5 vs 483.0 pg/mL, $P$=0.048, online supplemental table 3).

### Association of maternal vitamin B12 concentration with preterm birth

No evidence of the association was found between maternal vitamin B12 concentration and risk of preterm birth (per 100 pg/mL increment: adjusted HR (aHR), 0.99; 95% CI, 0.96 to 1.02, $P$=0.572 after adjustment for confounders) (table 2). No significant association was found when vitamin B12 concentration was categorised

Table 2 Association of maternal serum vitamin B12 concentration with the risk of preterm birth

| Variable | Preterm birth (%) | Model 1* | | Model 2 † | |
|---|---|---|---|---|---|
| | | HR (95% CI) | *P* value | aHR (95% CI) | *P* value |
| **Maternal serum vitamin B12 concentration ‡** | | | | | |
| Continuous, per 100 pg/mL | 1599 (5.9) | 0.99 (0.96,1.01) | 0.220 | 0.99 (0.96,1.02) | 0.572 |
| Clinical threshold | | | | | |
| <300 pg/mL | 206 (6.2) | 1.07 (0.92,1.24) | 0.363 | 1.03 (0.84,1.26) | 0.808 |
| ≥300 pg/mL | 1393 (5.9) | Reference | | Reference | |

All 95% CIs of HRs were not adjusted for multiple comparisons.
*Model 1 was adjusted for the duration between enrollment and conception (continuous).
†Model 2 was further adjusted for maternal age over 35 (binary), preconception overweight/obese (binary), parity (binary), smoking exposure (binary), drinking (binary), multivitamin supplementation (binary), GDM (binary) and HDP (binary) based on Model 1.
‡To convert vitamin B12 concentration from pg/mL to pmol/L, multiply by 0.7378.
HR, hazard ratio; CI, confidence interval.

according to the clinical thresholds (table 2). Similarly, null associations were observed for preterm subtypes (per 100 pg/mL increment; aHR, 0.98 [0.89 to 1.09] and 0.97 [0.90 to 1.05] for preterm birth<32 and<34 weeks, respectively) (table 3). Sensitivity analyses restricted to participants with complete vitamin B12 data or accounting for districts clustering all yielded similar findings (online supplemental tables 5-7).

RCS regression did not reveal any evidence of non-linear relationship between maternal vitamin B12 and preterm birth (*P* for non-linear=0.253, figure 2) as well as its subtypes (*P* for non-linear all >0.05, online supplemental figure 3).

## Subgroup analyses

The null association persisted among women enrolled before conception (aHR=0.99 [0.93 to 1.05] in online supplemental table 8) and those enrolled at early gestation (aHR=0.99 [0.95 to 1.04] in online supplemental table 9). Analyses using thresholds for the general population or pregnant women in the first trimester produced similar results (online supplemental tables 8, 9).

A decrease in vitamin B12 concentration was noted from preconception to early gestation, with an overall median decline of 78.0 pg/mL. Reductions were 8.0 pg/mL for women with preconception vitamin B12 levels <300 pg/mL vs 91.0 pg/mL for those with ≥300 pg/mL. However, null associations were found between changes in vitamin B12

Table 3 Association of maternal serum vitamin B12 concentration with the risk of preterm birth subtypes

| Variable | Preterm birth (%) | Model 1* | | Model 2 † | |
|---|---|---|---|---|---|
| | | HR (95% CI) | *P* value | aHR (95% CI) | *P* value |
| **Maternal serum vitamin B12 concentration ‡** | | | | | |
| **Continuous, per 100 pg/mL** | | | | | |
| Preterm birth <32 weeks | 170 (0.6) | 0.98 (0.91,1.06) | 0.627 | 0.98 (0.89,1.09) | 0.723 |
| Preterm birth <34 weeks | 339 (1.3) | 0.98 (0.93,1.03) | 0.436 | 0.97 (0.90,1.05) | 0.481 |
| **Clinical threshold** | | | | | |
| Preterm birth <32 weeks | | | | | |
| <300 pg/mL | 22 (0.7) | 1.07 (0.68,1.67) | 0.777 | 0.96 (0.51,1.81) | 0.894 |
| ≥300 pg/mL | 148 (0.6) | Reference | | Reference | |
| Preterm birth <34 weeks | | | | | |
| <300 pg/mL | 44 (1.3) | 1.07 (0.78,1.47) | 0.675 | 0.96 (0.62,1.51) | 0.875 |
| ≥300 pg/mL | 295 (1.2) | Reference | | Reference | |

All 95% CIs of HRs were not adjusted for multiple comparisons.
*Model 1 was adjusted for the duration between enrollment and conception (continuous).
†Model 2 was further adjusted for maternal age over 35 (binary), preconception overweight/obese (binary), parity (binary), smoking exposure (binary), drinking (binary), multivitamin supplementation (binary), GDM (binary) and HDP (binary) based on Model 1.
‡To convert vitamin B12 concentration from pg/mL to pmol/L, multiply by 0.7378.
HR, hazard ratio; CI, confidence interval.

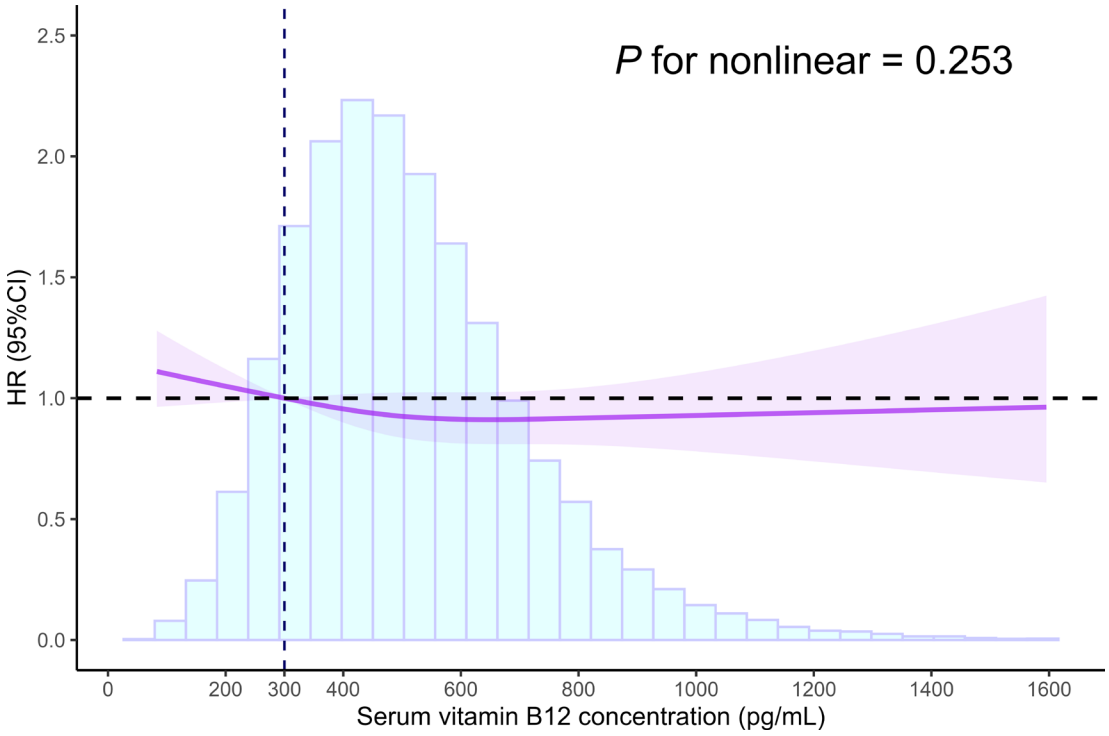


**Figure 2** Association of maternal vitamin B12 concentration with preterm birth using restricted cubic splines. The purple solid line indicates the estimated HR, and the shaded areas represent 95% CI. The sky-blue histogram shows the distribution of maternal serum vitamin B12 concentration in this study population, and the thresholds for vitamin B12 sufficiency (≥300 pg/mL) are indicated by a dashed line. To convert vitamin B12 concentration from pg/mL to pmol/L, multiply by 0.7378. The model was adjusted for the duration between enrolment and conception (continuous), maternal age over 35 (binary), preconception overweight/obese (binary), parity (binary), smoking exposure (binary), drinking (binary), multivitamin supplementation (binary), GDM (binary) and HDP (binary). The RCS did not show evidence of non-linear associations between maternal vitamin B12 concentration and preterm birth (*P* for non-linear=0.253).

concentration and risk of preterm birth (online supplemental table 10).

## DISCUSSION

In this study, no evidence shows associations between maternal vitamin B12 concentration and the risk of preterm birth as well as its subtypes among Chinese women, the majority of whom had sufficient vitamin B12 levels. Neither vitamin B12 deficiency nor changes in vitamin B12 levels from preconception to early gestation were associated with the risk of preterm birth.

Vitamin B12 is integral to one-carbon metabolism, critical for DNA synthesis, methylation and mitochondrial function[14] and is therefore essential for embryonic development and maternal–child health.[15 28] During pregnancy, the demand for vitamin B12 increases due to foetal growth, maternal blood volume expansion and tissue development.[29 30] Inadequate maternal vitamin B12 levels during the periconception period may negatively affect birth outcomes and long-term offspring health, including cardiometabolic health,[30] insulin resistance and neuropsychological development.[31 32]

Previous studies on the association between maternal vitamin B12 concentration and preterm birth were limited and inconclusive. Most studies have reported a reduced risk of preterm birth with higher maternal vitamin B12 concentrations, whereas others have found null or opposite associations. A meta-analysis consisting of 18 worldwide cohort studies by Rogne et al[18] found a linear association between maternal serum or plasma B12 concentration during pregnancy and reduced risk of preterm birth, with each SD increment in vitamin B12 associated with an 11% (adjusted relative risk [aRR]=0.89 [0.82–0.97]) reduced risk of preterm birth, and vitamin B12 deficiency linked to a 21% (aRR=1.21 [0.99–1.49]) increased risk. The pooled population had mean vitamin B12 concentrations of 219.8, 187.8 and 188.7 pmol/L in the first, second and third trimesters, respectively, and the median vitamin B12 deficiency rate was 33%. However, our study population had a median vitamin B12 concentration of 483.0 pg/mL (equivalent to 356.4 pmol/L), with lower proportions of deficiency and marginal deficiency only at 2.4% and 10.0%, respectively. Liu et al[33] reported similar findings in their case–control study in Sichuan Province, China, with a comparable median serum vitamin B12 concentration at early gestation (401.28 pmol/L). In addition, the other two studies reported differing results. Chen et al[19] found no significant association between maternal plasma vitamin B12 concentration during 26–28 weeks of gestation and preterm birth, with

a median concentration of 209 pmol/L. Dhobale et al[20] reported higher plasma vitamin B12 levels before delivery in preterm birth cases. Differences in study design, timing of vitamin B12 measurement and population heterogeneity of vitamin B12 levels might account for the discrepancies across studies. Notably, vitamin B12 concentrations in our cohort were relatively higher than those reported in most previous studies, which may partly explain the weaker effects observed. This aligns with the epidemiological concept that higher baseline levels of exposure may attenuate the observed effects. It is reasonable to assume that the association between maternal vitamin B12 and preterm birth might not be evident among populations with relatively sufficient serum vitamin B12 levels. Furthermore, the current effect sizes were smaller than those in our previous study on vitamin B12 and GDM in the same population,[34] indicating limited clinical relevance for preterm birth.

Additionally, maternal vitamin B12 concentrations naturally decrease during pregnancy due to haemodilution and preferential transfer of vitamin B12 to the foetus.[17] Schroder et al[27] suggested new reference intervals for serum vitamin B12 in the first trimester in a previous study with vitamin B12 <89.9 pmol/L (equivalent to <122 pg/mL) for deficiency and <186 pmol/L (equivalent to <252 pg/mL) for impaired intracellular B12 status, which were much lower than the commonly used thresholds for the general population. However, we did not find evidence of an association between vitamin B12 deficiency and preterm birth according to either the thresholds for the general population or the new thresholds for pregnant women at early gestation, nor did we find an association for the changes in vitamin B12 concentration from preconception to early gestation.

Despite the overall null findings, this study contributes novel evidence by exploring subtype-specific associations and the impact of preconception vitamin B12 levels on preterm birth risk, areas that have not been previously examined in a large Chinese cohort. However, our findings should be interpreted with caution. We cannot simply deny the potential protective effect of sufficient vitamin B12 levels on populations with poor vitamin B12 levels, where supplementation might yield a more pronounced benefit. Further research among the population of diverse baseline levels of vitamin B12 is needed for a better understanding of the potential public health benefits of vitamin B12 supplementation in periconception care.

Several mechanisms might link vitamin B12 to preterm birth, though they are not fully understood. One potential mechanism involves inflammation, a known cause of preterm birth, resulting from an imbalance between maternal inflammation and hormonal-driven uterine quiescence.[3 35] Studies have shown differences in decidual inflammatory signalling and cytokine levels between term and preterm births.[35–37] Animal studies also suggest that folic acid may protect against lipopolysaccharide-induced preterm delivery through anti-inflammatory effects.[38] Another mechanism involves DNA methylation, as vitamin B12 is essential for DNA synthesis and methylation, processes that are involved in parturition and preterm birth.[39] Molecular studies indicate that one-carbon metabolism supports inflammatory macrophages through S-adenosylmethionine and histone methylation.[40] However, the current study did not support these hypothesised pathways, and further studies are needed.

To our knowledge, this is the first large-scale prospective study to investigate the association of maternal periconception serum vitamin B12, particularly levels before conception, with preterm birth and its subtypes in such a large Chinese cohort. The strengths of this study include its large population-based prospective cohort design, the objective measurement of vitamin B12 starting from before conception. However, several limitations should be considered. First, potential selection bias may exist as participants enrolled in our cohort tend to be more health-conscious, the majority achieved sufficient vitamin B12 levels and our findings may not be generalised to populations with insufficient or excessive vitamin B12 levels. Second, misclassification bias could have occurred in the outcome measurement due to challenges in distinguishing iatrogenic preterm birth, as detailed medical records were unavailable. Third, bias from unmeasured confounders cannot be entirely ruled out, such as the effect of dietary intake of vitamin B12 from food consumption, placenta previa or iatrogenic preterm birth due to the lack of available data. Fourth, other biomarkers related to vitamin B12 status, such as methylmalonic acid, were not measured, which limits the ability to fully interpret our findings. Lastly, our findings may not be directly generalised to populations from regions with food fortification or with population with low levels of vitamin B12.

## CONCLUSION

In this study, no evidence shows an association between maternal vitamin B12 concentration and the risk of preterm birth in the study population, in which the majority achieved sufficient vitamin B12 levels. However, the clinical and public health implications of the findings should be interpreted with caution, particularly for women with low vitamin B12 levels, where warrant recommendations of ensuring adequate micronutrients through supplementation or foods as a preventative strategy. These results contribute novel, population-based evidence to the growing body of association between maternal vitamin B12 and preterm birth, especially in the context of preconception. Further studies in populations with diverse baseline levels of vitamin B12 are needed to explore underlying mechanisms and validate these findings in different populations and regions.

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**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and was approved by the Institutional Review Board at the Children's Hospital of Fudan University (IRB number 201649). Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available upon reasonable request. The data that support the findings of this study are available from the corresponding author upon reasonable request.



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