

Maternal Serum Zinc Concentration during Pregnancy Is Inversely Associated with Risk of Preterm Birth in a Chinese Population^{1–3}

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Abstract

Background: Evidence exists that maternal zinc status during pregnancy is linked to adverse pregnancy outcomes including abortion, fetal growth restriction, and neural tube defects. However, it remains unclear whether maternal serum zinc concentration (SZC) during pregnancy is associated with risk of preterm birth.

Objective: This study was designed to investigate the association between maternal SZC during pregnancy and risk of preterm birth.

Methods: For this substudy of the China-Anhui Birth Cohort Study, 3081 maternal-singleton pairs with detailed birth records and available serum samples were identified. The maternal SZC was determined with flame atomic absorption spectroscopy. A total of 169 preterm births were identified. In this study, the women were divided into tertiles on the basis of their SZC: low (<76.7 $\mu\text{g/dL}$), medium (76.7–99.6 $\mu\text{g/dL}$), and high (≥ 99.7 $\mu\text{g/dL}$). The ORs for preterm birth were estimated by using multiple logistic regression models.

Results: The median SZC was 87.3 $\mu\text{g/dL}$ (range: 11.1–211 $\mu\text{g/dL}$). Incidences of preterm birth were 7.3% and 6.0% among subjects with low and medium SZCs, respectively, which were significantly higher than 3.1% among subjects with a high SZC [ORs (95% CIs) for low and medium SZCs: 2.45 (1.60, 3.74), $P < 0.001$, and 2.00 (1.29, 3.09), $P < 0.01$, respectively]. After adjustment for prepregnancy body mass index, maternal age, time of serum collection, gravidity, parity, and monthly income, adjusted ORs were 2.41 (95% CI: 1.57, 3.70; $P < 0.001$) and 1.97 (95% CI: 1.27, 3.05; $P < 0.01$) among subjects with low and medium maternal SZCs.

Conclusions: Maternal serum zinc concentration during pregnancy is inversely associated with risk of preterm birth in the Chinese population, and the results are driven by maternal SZC in the first trimester. *J Nutr* 2016;146:509–15.

Keywords: maternal serum, zinc, preterm birth, pregnancy, birth cohort study

Introduction

Preterm birth, defined as spontaneous or iatrogenic birth before gestational week 37, is a major reason for neonatal death (1, 2). Numerous epidemiologic studies have shown an association between preterm birth and childhood asthma (3, 4). Moreover,

preterm birth increases the risk of cardiovascular diseases in young adulthood (5, 6) and is an important independent risk factor for neurodevelopmental disorders (7). Although maternal obesity, maternal inflammation, and accumulation of nonesterified FAs in maternal serum have been identified as potential risk factors (8–10), the exact etiology and underlying mechanisms for preterm birth remain obscure.

Zinc is a structural constituent that is essential for cell growth, cell differentiation, and development (11). Several earlier reports showed that low maternal zinc concentration during pregnancy is associated with adverse pregnancy outcomes, including abortion, stillbirth, and fetal neural tube defects (12–14). Moreover, the association between maternal zinc insufficiency during pregnancy and intrauterine fetal growth restriction has been investigated in several small epidemiologic studies (15, 16). However, it remains unclear whether maternal serum zinc concentration (SZC) during pregnancy is associated with the risk of preterm birth. According to

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³ Supplemental Table 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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a small birth cohort study, maternal SZC during pregnancy was associated with an increased risk of preterm delivery (17). A 2015 meta-analysis showed that maternal zinc supplementation caused a 14% relative reduction in preterm births (18). However, a randomized controlled trial found that maternal zinc supplementation did not affect pregnancy duration (19). This result was in agreement with a systematic review, in which the evidence that maternal zinc supplementation reduced the risk of preterm birth was low (20). Thus, whether maternal SZC during pregnancy is associated with risk of preterm birth needs to be further determined. The objective of the present study was to investigate the association between maternal SZC during pregnancy and the risk of preterm birth in a Chinese population.

Methods

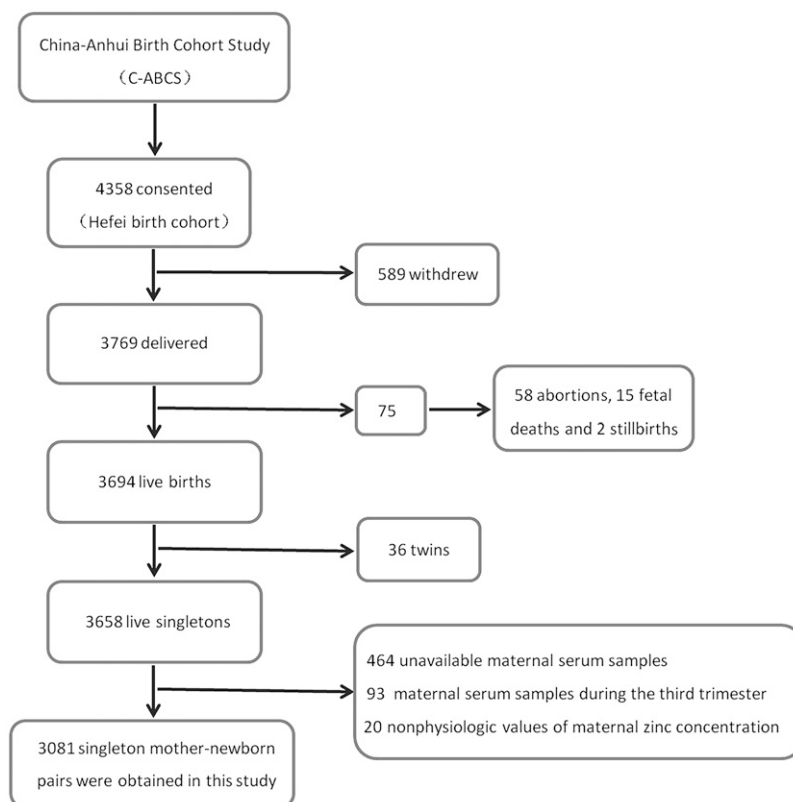
Participant recruitment. The China-Anhui Birth Cohort Study is a prospective, population-based cohort study that recruited 16,766 pregnant women from 6 major cities of Anhui province in China between November 2008 and October 2010. A total of 13,454 singleton live births were followed up from this cohort (21). The present study analyzed a substudy of the China-Anhui Birth Cohort Study that recruited 4358 pregnant women from Hefei, a city in Anhui province, from 1 January to 31 December 2009. Exclusion criteria for participation were as follows: inability to provide informed consent, alcohol drinking and cigarette smoking during pregnancy, mental disorders, pregnancy-induced hypertension and pre-eclampsia, gestational diabetes, heart disease, thyroid-related disease, a history of ≥ 3 previous miscarriages, or plans to leave the area before delivery. For this study, eligible participants were mother and singleton offspring pairs in which serum samples from mothers were available for analysis of SZC and offspring had detailed birth records. In this substudy, 36 pregnant women gave birth to twins, there were 15 fetal deaths, 2 stillbirths, 58 abortions, and 589

withdrew from the study (Figure 1). In addition, 464 participants with no maternal sera available, 20 with nonphysiologically plausible SZC values, and 93 with samples collected in the third trimester were also excluded (Figure 1). As a result of differences in time at entry into our cohort, maternal serum samples were collected from 1069 pregnant women in the first trimester (4–12 wk of gestation) and 2012 samples were collected from pregnant women in the second trimester (13–27 wk of gestation). The present study obtained ethical approval from the Human Research Ethics Committee of Anhui Medical University (permit 08-1026). Oral and written consent was obtained from all pregnant women.

Definition of preterm birth. Gestational age was calculated by using the mother's last menstrual period. Preterm birth was defined as a live birth at < 37 completed gestational weeks (22). Deliveries at < 28 wk of gestational age were considered miscarriages and excluded in the current analysis (23). A total of 169 spontaneous and nonmedical preterm deliveries were identified.

Measurement of maternal SZC. Maternal fasting blood during pregnancy was collected in the morning. The blood samples were allowed to clot at room temperature for 30 min. Maternal serum was then obtained after centrifuging for 15 min at 3000 g. After discarding hemolytic specimens, available sera were stored at -80°C until analysis. SZC was determined by flame atomic absorption spectroscopy as previously described (24). Briefly, all tubes and pipette tips were immersed overnight in 10% HNO_3 at room temperature. Serum samples were diluted with 1% HNO_3 at 1:35 (vol:vol). The diluted solution was then detected by using flame atomic absorption spectroscopy. Each sample was analyzed in triplicate. The precision of the method was measured by using CVs. The mean CV for measurement of serum zinc was 4.9% for within-day determinations and 4.3% for day-to-day determinations. The detection limit of this method was 0.2 $\mu\text{g}/\text{dL}$. According to a method described by a 2014 study (25), the pregnant women were divided into tertiles on the basis of their SZC and classified as having low (< 76.7 $\mu\text{g}/\text{dL}$), medium (76.7–99.6 $\mu\text{g}/\text{dL}$), or high (≥ 99.7 $\mu\text{g}/\text{dL}$) SZCs.

FIGURE 1 Flow diagram of recruitment, follow-up, and identification of serum zinc in a substudy of the China-Anhui Birth Cohort Study.



Confounding factors. According to a previous systemic review (26), potential confounding factors that might influence the association between maternal zinc status during pregnancy and preterm birth were chosen as follows: maternal age (≤ 24 , 25–29, or ≥ 30 y), prepregnancy BMI (in kg/m^2 ; < 18.5 , 18.5–24.9, or ≥ 25), average monthly income (low income: < 2000 yuan renminbi or 312 US\$; middle income: 2000–3999 yuan renminbi or 312–624 US\$; high income: ≥ 4000 yuan renminbi or 624 US\$), time of serum collection [first trimester: median, 11 wk of gestation (range: 4–12 wk of gestation); second trimester: median, 16 wk of gestation (range: 13–27 wk of gestation)], gravidity (primigravida or multigravida) and parity (nulliparous or multiparous), and multivitamin intake (yes or no).

Statistical analysis. First, the ratios of maternal and neonatal characters, and the rate of preterm birth among tertiles of maternal SZC, were analyzed by using the chi-square test. Continuous measures, including SZC, were compared between 2 groups by using independent-samples *t* tests. For multiple comparisons, we used 1-factor ANOVA followed by Bonferroni's or Tamhane's T2 post hoc test. Next, ORs and 95% CIs for the association between maternal zinc status and preterm birth were estimated by using multiple logistic regression models. We calculated unadjusted and adjusted estimates using exact methods and asymptotic methods, respectively. To identify other variables for

inclusion in the multivariable models, we sequentially tested each maternal characteristic listed in Table 1 in the basic model, which included maternal age, prepregnancy BMI, monthly income, time of serum collection, gravidity, and parity. For any tertile of maternal SZC, 3 covariates (pregnancy BMI, gravidity, and parity) changed the adjusted OR for the association with preterm birth by $> 10\%$ (Table 2) (27). Because of the low incidence of preterm birth, the OR is a good approximation of the relative risk (28). We performed all statistical analyses with SPSS statistical software (version 16.0). All statistical tests were 2-sided with an α level of 0.05.

Results

Demographic characteristics of pregnant women. Demographic characteristics of the pregnant women are presented in Table 1. No significant differences in maternal age, prepregnancy BMI, gravidity, or monthly income were observed between subjects with low, medium, and high maternal SZCs. With decreasing tertiles of maternal SZC, the proportion of serum samples collected in the first trimester decreased, whereas the percentages of multipara both increased. The characteristics of newborns were then analyzed. As shown in Table 1, the rates

TABLE 1 Characteristics of 3081 mothers and their newborns according to maternal serum zinc concentration in a Chinese population¹

	Maternal serum zinc concentration			P
	Low ($< 76.7 \mu\text{g}/\text{dL}$)	Medium (76.7–99.6 $\mu\text{g}/\text{dL}$)	High ($\geq 99.7 \mu\text{g}/\text{dL}$)	
<i>n</i>	1027	1027	1027	
Maternal characteristics				
Age, y	27.4 \pm 3.3	27.5 \pm 3.2	27.5 \pm 3.0	0.97
≤ 24 y, <i>n</i> (%)	163 (15.9)	147 (14.3)	161 (15.7)	0.82
25–29 y, <i>n</i> (%)	646 (62.9)	665 (64.8)	643 (62.6)	
≥ 30 y, <i>n</i> (%)	218 (21.2)	215 (20.9)	223 (21.7)	
Prepregnancy BMI, kg/m^2	20.1 \pm 2.3	20.2 \pm 2.2	20.3 \pm 2.1	0.36
$< 18.5 \text{ kg}/\text{m}^2$, <i>n</i> (%)	247 (24.1)	220 (21.4)	192 (18.7)	< 0.01
18.5–24.9 kg/m^2 , <i>n</i> (%)	740 (72.1)	776 (75.6)	810 (78.9)	
$\geq 25 \text{ kg}/\text{m}^2$, <i>n</i> (%)	40 (3.9)	31 (3.0)	25 (2.4)	
Gravidity, <i>n</i> (%)				0.77
Primigravida	530 (51.6)	545 (53.1)	532 (51.8)	
Multigravida	497 (48.4)	482 (46.9)	495 (48.2)	
Parity, <i>n</i> (%)				0.04
Nulliparous	988 (96.2)	999 (97.3)	1007 (98.1)	
Multiparous	39 (3.8)	28 (2.7)	20 (1.9)	
Monthly income, ² <i>n</i> (%)				0.38
Low	482 (46.9)	449 (43.7)	475 (46.3)	
Middle	412 (40.1)	441 (42.9)	403 (39.2)	
High	133 (13.0)	137 (13.3)	149 (14.5)	
Time of serum collection, wk	15.8 \pm 4.5	14.6 \pm 4.2	14.0 \pm 4.3	< 0.001
First trimester, <i>n</i> (%)	247 (24.1)	364 (35.4)	458 (44.6)	< 0.001
Second trimester, <i>n</i> (%)	780 (75.9)	663 (64.6)	569 (55.4)	
Newborn characteristics				
Gestational age, wk	38.9 \pm 1.8	39.0 \pm 1.8	39.1 \pm 1.6	0.012
< 37 wk, <i>n</i> (%)	75 (7.3)	62 (6.0)	32 (3.1)	< 0.001
≥ 37 wk, <i>n</i> (%)	952 (92.7)	965 (94.0)	995 (96.9)	
Birth weight, g	3378 \pm 483	3403 \pm 446	3413 \pm 452	0.21
< 2500 g, <i>n</i> (%)	38 (3.7)	25 (2.4)	17 (1.7)	0.013
≥ 2500 g, <i>n</i> (%)	989 (96.3)	1002 (97.6)	1010 (98.3)	

¹ Values are means \pm SDs unless otherwise indicated.

² Low income was < 2000 yuan renminbi (312 US\$) per month; middle income was 2000–3999 yuan renminbi (312–624 US\$) per month; and high income was ≥ 4000 yuan renminbi (624 US\$) per month.

TABLE 2 Associations of maternal characteristics with risk of preterm birth in a Chinese population

Maternal characteristics	n (%)	Preterm birth	
		OR (95% CI)	P
Age			
≤24 y	471 (15.3)	0.99 (0.63, 1.55)	0.96
25–29 y	1954 (63.4)	1.00	—
≥30 y	656 (21.3)	1.11 (0.76, 1.63)	0.58
Prepregnancy BMI (in kg/m ²)			
<18.5	659 (21.4)	1.38 (0.96, 1.97)	0.08
18.5–24.9	2326 (75.5)	1.00	—
≥25	96 (3.1)	2.24 (1.13, 4.42)	0.02
Gravidity			
Primigravida	1607 (52.2)	1.00	—
Multigravida	1474 (47.8)	0.80 (0.58, 1.09)	0.16
Parity			
Nulliparous	2994 (97.2)	1.00	—
Multiparous	87 (2.8)	1.78 (0.85, 3.75)	0.13
Monthly income ¹			
Low	1406 (45.6)	0.90 (0.56, 1.45)	0.67
Middle	1256 (40.8)	1.00 (0.62, 1.61)	0.99
High	419 (13.6)	1.00	—
Time of serum collection			
First trimester	1069 (34.7)	1.00	—
Second trimester	2012 (65.3)	1.14 (0.82, 1.59)	0.44

¹ Low income was <2000 yuan renminbi (312 US\$) per month; middle income was 2000–3999 yuan renminbi (312–624 US\$) per month; and high income was ≥4000 yuan renminbi (624 US\$) per month.

of preterm birth and low birth weight gradually increased with decreasing tertiles of maternal SZC.

Maternal SZC during pregnancy and risk of preterm birth. The median SZC was 87.3 µg/dL. Several maternal characteristics, including age, monthly income, gravidity, and multivitamin intake, were not associated with SZC during pregnancy (Supplemental Table 1). Of interest, the mean SZC was significantly lower among subjects with a prepregnancy BMI <18.5 than that in subjects with a normal BMI (18.5–24.9). In addition, the mean SZC among nulliparae was significantly higher than that in multiparae. Subsequently, the mean SZC among different gestational ages was further analyzed (Supplemental Table 1). Results showed that the mean SZC among subjects with 33.0–34.9 and 35.0–36.9 wk of gestation was markedly lower than that in subjects with ≥37.0 wk of gestation (Supplemental Table 1). The associations of other known risk factors and preterm birth were further analyzed. As shown in

Table 2, some risk factors, including maternal age, monthly income, gravidity, and prepregnancy BMI, were not associated with the risk of preterm birth.

As shown in Table 3, the incidences of preterm birth among the low-, medium-, and high-zinc groups were 7.3%, 6.0%, and 3.1%, respectively. Subjects with low and medium maternal SZCs had a significantly higher risk of preterm birth than did those with a high maternal SZC. After adjusting for maternal age, prepregnancy BMI, time of serum collection, monthly income, parity, and gravidity, the ORs for preterm birth were 2.41 and 1.97 among subjects with low and medium maternal SZCs, respectively (Table 3).

Maternal SZC in the first trimester and risk of preterm birth. As shown in Table 4, the incidences of preterm birth were 8.1% and 7.1% among subjects with low and medium first-trimester SZCs, which was significantly higher than the 1.7% incidence among subjects with a high SZC. After adjusting for prepregnancy BMI, maternal age, monthly income, parity, and gravidity, the ORs for preterm birth were 4.90 and 4.35 among subjects with low and medium SZCs, respectively (Table 4).

Maternal SZC in the second trimester and risk of preterm birth. The incidences of preterm birth among the low-, medium-, and high-zinc groups were 7.1%, 5.4%, and 4.2%, respectively (Table 5). No significant difference in risk of preterm birth was observed among subjects by SZC in the second trimester.

Discussion

The incidence of preterm birth was 5.5% in our birth cohort and was significantly lower than 11.1% (uncertainty range: 9.1–13.4%), which is the global average preterm birth rate (13% in India and 12.0% in the United States) (29). Our study, however, excluded medically indicated preterm deliveries and miscarriages (<28 wk).

According to several systemic reviews, maternal zinc supplementation resulted in a 14% relative reduction in preterm births (18, 30, 31). To analyze the association between maternal SZC during pregnancy and the risk of preterm birth, the present study calculated the incidence and OR of preterm birth among 3 groups on the basis of tertiles of maternal SZC. Results showed that the incidences of preterm birth in low-, medium-, and high-zinc groups were 7.3%, 6.0%, and 3.1%, respectively. Subjects with low and medium SZCs had a significantly higher risk of preterm birth than did those in the high-SZC group. These results are in agreement with an earlier

TABLE 3 Incidences and ORs of preterm birth on the basis of maternal serum zinc concentration during pregnancy in a Chinese population¹

	Maternal serum zinc concentration			P
	Low (<76.7 µg/dL)	Medium (76.7– 99.6 µg/dL)	High (≥99.7 µg/dL)	
Newborns, n	1027	1027	1027	
Preterm births, n	75	62	32	
Incidence, %	7.3	6.0	3.1	<0.001
Univariate OR (95% CI)	2.45 ^b (1.60, 3.74)	2.00 ^a (1.29, 3.09)	1.00	<0.001
Adjusted OR (95% CI) ²	2.41 ^b (1.57, 3.70)	1.97 ^a (1.27, 3.05)	1.00	<0.001

¹a,b Different from reference (high maternal zinc concentration): ^aP < 0.01, ^bP < 0.001.

² Adjusted for prepregnancy BMI, maternal age, time of serum collection, gravidity, parity, and monthly income.

TABLE 4 Incidences and ORs of preterm birth on the basis of maternal serum zinc concentration during the first trimester in a Chinese population¹

	Maternal serum zinc concentration			<i>P</i>
	Low (<76.7 μg/dL)	Medium (76.7–99.6 μg/dL)	High (≥99.7 μg/dL)	
Newborns, <i>n</i>	247	364	458	
Preterm births, <i>n</i>	20	26	8	
Incidence, %	8.1	7.1	1.7	<0.001
Univariate OR (95% CI)	4.96 ^b (2.15, 11.43)	4.33 ^b (1.94, 9.68)	1.00	<0.001
Adjusted OR (95% CI) ²	4.90 ^b (2.12, 11.32)	4.35 ^b (1.94, 9.74)	1.00	<0.001

^{1b} Different from reference (high maternal zinc concentration), *P* < 0.001.

² Adjusted for prepregnancy BMI, maternal age, gravidity, parity, and monthly income.

report from a small birth cohort study, in which low zinc concentration during pregnancy was associated with an increased risk of preterm birth (17). Together, these results suggest that maternal zinc concentration during pregnancy is negatively associated with the risk of preterm birth.

To our knowledge, until now, no report has analyzed the association between maternal zinc status at different gestational ages and the risk of preterm birth. The present study compared differential effects of maternal zinc concentration at early and middle gestational ages on the incidence of preterm birth. Unexpectedly, the incidence of preterm infants was 8.0% among subjects with a low SZC in the first trimester, which is significantly higher than 1.7% among those with a high SZC in the first trimester. Interestingly, the incidence of preterm births was 7.0% among subjects with a low maternal SZC in the second trimester, which was not significantly higher than the incidence of 4.4% among those with a high maternal SZC in the second trimester. These results suggest that maternal zinc status in the first trimester but not middle gestational age is negatively associated with the risk of preterm birth.

The mechanism by which maternal zinc insufficiency during pregnancy elevates the risk of preterm birth remains obscure. Increasing evidence has shown that zinc has anti-inflammatory activity (32). An earlier study found that zinc supplementation alleviated early inflammatory responses during cutaneous wound healing (33). Another study showed that zinc supplementation reduced the number of eosinophils in bronchoalveolar lavage fluid during allergic inflammation (34). According to a small, double-blind, randomized controlled trial, zinc supplementation markedly improved cell-mediated immunity and anti-inflammatory activity in children (35). In an earlier study, we showed that zinc supplementation alleviated LPS-induced placental inflammation

(36), and placental inflammation is associated with preterm birth (37–39). Several studies showed that inflammatory cytokines were elevated in cervicovaginal fluid, vaginal fluid, and amniotic fluid in women delivering preterm infants (40–43). Taken together, these results suggest that there might be an association between maternal zinc insufficiency during pregnancy, maternal inflammation, and the risk of preterm birth.

Zinc may be used as a potential protective and therapeutic agent for clinical therapy, especially in high-risk situations in which pregnant women are infected with bacteria. According to a study from our laboratory, oral zinc supplementation ameliorated LPS-induced intrauterine fetal growth restriction (36). Additional study is required to determine whether maternal zinc supplementation during pregnancy reduces the risk of preterm birth in randomized placebo-controlled trials.

The present study has some limitations. First, we did not explore the reasons for low maternal SZC. Second, preterm birth was classified according to menstrual period-based pregnancy dating, not ultrasound-based pregnancy dating. Third, the present study did not have any data on some environmental factors that may be important for preterm birth. However, many lifestyle factors and nutritional status that influence preterm birth are driven by socioeconomic status (26). This study controlled for socioeconomic status (monthly income) as a confounding factor, which may have reduced the bias. Next, we did not adjust for maternal insufficiency of other micronutrients, such as folate and vitamin D, which also elevate the risk of preterm delivery (44, 45). In summary, the present study shows that maternal SZC during pregnancy is inversely associated with the risk of preterm birth, and the results are driven by maternal SZC in the first trimester.

TABLE 5 Incidences and ORs of preterm birth on the basis of maternal serum zinc concentration during the second trimester in a Chinese population

	Maternal serum zinc concentration			<i>P</i>
	Low (<76.7 μg/dL)	Medium (76.7–99.6 μg/dL)	High (≥99.7 μg/dL)	
Newborns, <i>n</i>	780	663	569	
Preterm births, <i>n</i>	55	36	24	
Incidence, %	7.1	5.4	4.2	0.08
Univariate OR (95% CI)	1.72 (1.05, 2.82)	1.30 (0.77, 2.21)	1.00	0.08
Adjusted OR (95% CI) ¹	1.68 (1.02, 2.75)	1.28 (0.75, 2.18)	1.00	0.10

¹ Adjusted for prepregnancy BMI, maternal age, gravidity, parity, and monthly income.

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