Environment International 122 (2019) 237–243

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Association of maternal serum copper during early pregnancy with the risk of spontaneous preterm birth: A nested case-control study in China

Yongxiu Hao[a,b], Yiming Pang[a,b], Huina Yan[a,b], Yali Zhang[a,b], Jufen Liu[a,b], Lei Jin[a,b], Lailai Yan[c], Da Chen[d], Ligang Hu[e], Qian Liu[e], Yanan Qin[a,b], Bin Wang[a,b], Zhiwen Li[a,b], Rongwei Ye[a,b,*], Le Zhang[a,b,*], Aiguo Ren[a,b]

[a] Institute of Reproductive and Child Health, Peking University/Key Laboratory of Reproductive Health, National Health Commission of the People's Republic of China, Beijing 100191, PR China
[b] Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing 100191, PR China
[c] Department of Laboratorial Science and Technology, School of Public Health, Peking University, Beijing 100191, PR China
[d] School of Environment, Jinan University, Guangdong 510632, PR China
[e] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, PR China

ARTICLE INFO

Handling Editor: Yong-Guan Zhu

Keywords:
Copper
Spontaneous preterm birth
Cholesterol
Triglycerides
Pregnancy

ABSTRACT

Background: The effect of maternal copper (Cu) level on the risk of spontaneous preterm birth (SPB) remains debate. Therefore, we conducted a prospective nested case-control study in Shanxi Province to investigate the relationship between maternal serum Cu concentration and SPB risk, as well as the potential mediation effect of lipid metabolism.

Method: From an overall cohort of 4229 women, 147 women affected by SPB at 20–36 gestational weeks (cases) and 381 women who delivered at ≥37 gestational weeks (controls) were included in our nested case-control study. Maternal blood samples were collected during 4–22 gestational weeks, and the concentrations of Cu, total cholesterol (TC), and triglycerides (TG) were measured. Information on maternal social demographic characteristics were collected using questionnaires. Unconditional logistic regression models were used to estimate the associations of Cu, TC or TG levels with SPB risk. Linear regressions were used to assess the relationships between concentrations of Cu and TC or TG.

Results: Serum Cu concentrations in the case group (median: 184 μg/dL) were significantly higher than those in the control group (median: 166 μg/dL, $p < 0.0001$). Compared to the lowest serum Cu levels, the odds ratios associated with SPB increased to 2.02 (95% confidence interval [CI]: 1.07, 3.82), 3.10 (1.54, 6.22) and 4.18 (2.11, 8.27) in the second, third and fourth quartile respectively, after adjusting for sampling time, maternal age, pre-pregnancy BMI, education, occupation, parity, spontaneous abortion history, folic acid use, medication use, pre-pregnancy passive smoking status, child gender and fasting status. Plasma concentrations of TC and TG were positively associated with SPB risk in a dose-dependent manner. However, when stratified by sampling time, the above-mentioned relationships were significant in the first trimester but not in the second. In addition, plasma concentrations of TC and TG were positively correlated with serum Cu concentrations.

Conclusions: High maternal Cu level in the first trimester may increase the risk of SPB, by potentially increasing plasma concentrations of TC and TG.

1. Introduction

Preterm birth, defined as a live birth at < 37 weeks gestational age, remains one of the leading causes of perinatal morbidity and mortality worldwide (Liu et al., 2012). In China, about 7.1% of live births are affected by preterm birth (Blencowe et al., 2012), and about 75% of these are spontaneous preterm birth (SPB) (Zou et al., 2014), including spontaneous preterm labor with intact membranes and preterm premature rupture of the membranes (PPROM) (Goldenberg et al., 2008). Although risk factors for SPB (e.g., history of preterm birth, periodontal

* Corresponding authors at: Institute of Reproductive and Child Health, Peking University/Key Laboratory of Reproductive Health, National Health Commission of the People's Republic of China, Beijing 100191, PR China.
E-mail addresses: yerw@bjmu.edu.cn (R. Ye), zhangle@bjmu.edu.cn (L. Zhang).

https://doi.org/10.1016/j.envint.2018.11.009
Received 24 July 2018; Received in revised form 31 October 2018; Accepted 5 November 2018
Available online 22 November 2018
0160-4120/

disease, and low maternal body mass index) have been reported, the underlying drivers of SPB remain unclear. In particular, knowledge gaps exist on the effect of the imbalance of maternal essential trace metals on SPB.

Copper (Cu) is an essential trace element for numerous biological processes, but disorders of Cu metabolism in pregnancy, either deficiencies or excesses, can lead to severe diseases, such as preeclampsia (Song et al., 2017). However, effect of Cu intake on the risk of preterm birth is not well understood. Recently, some studies (Li et al., 2018; Perveen et al., 2002; Schulpis et al., 2004; Zadrożna et al., 2009) have suggested an increased risk of preterm birth with Cu deficiency and while some others (Alebic-Juretic and Frkovic, 2005; Carmichael et al., 2013) have found no association between Cu level and preterm birth. However, most studies (Alebic-Juretic and Frkovic, 2005; Carmichael et al., 2013; Li et al., 2018; Perveen et al., 2002; Schulpis et al., 2004) have only focused on overall preterm birth and have not separated iatrogenic preterm birth from SPB. However, iatrogenic preterm birth is the labor induction or caesarean delivery caused by maternal or fetal complications and for SPB the exact causes or mechanisms are still unclear (Goldenberg et al., 2008). They are different subtypes of preterm birth and involved with different risk factors and underlying mechanisms, so it is not suitable to take iatrogenic preterm birth and SPB as a whole when discuss their risk factors and mechanisms (Dekker et al., 2012; Goldenberg et al., 2008). Additionally, most previous studies (Perveen et al., 2002; Schulpis et al., 2004; Zadrożna et al., 2009) were case-control studies and the Cu concentrations were investigated in samples collected post delivery, such as umbilical cord blood (Li et al., 2018; Perveen et al., 2002), newborn blood (Schulpis et al., 2004) or placenta (Zadrożna et al., 2009). During pregnancy, Cu concentration increased with gestational age (Izquierdo et al., 2007). So in these studies the gestational age of sampling time was different between term birth ($\geq$37 weeks) and preterm birth ($<$ 37 weeks) and the difference of gestational age may cause a substantiality discrepancy of Cu concentration. In addition, from a preventative point of view, understanding the impact of maternal blood Cu level, especially during the first and second trimesters, is of particular interest, because it is relatively easy to intervene before delivery and may have more effective results on pregnancy outcomes. However, to the best of our knowledge, only one cohort study (Wilson et al., 2018) have examined the association between maternal Cu levels and the risk of SPB and suggested lower Cu levels were associated with lower risk of preterm birth in first trimester. But the sample size of that study was relative small (N = 1065). Therefore, it is necessary to investigate the relationship of maternal Cu intake and SPB risk at an early stage in study with a large sample size.

Some epidemiology (Shoji et al., 2017; Wells et al., 2014) and animal studies (Galhardi et al., 2004) have documented a positive relationship between Cu intake and dyslipidemia, including disorders of total cholesterol (TC) and triglycerides (TG) by increasing lipid hydroperoxide concentrations and reducing the superoxide dismutase and glutathione peroxidase activities. In addition, several studies have reported that the risk of SPB increases with high concentrations of TC and TG (Catov et al., 2008; Catov et al., 2007a, b; Jiang et al., 2017; Mudd et al., 2012). Therefore, we hypothesized that high maternal Cu intake levels will be associated with an increased risk of SPB via increases in the concentrations of TC and TG. Though, the mechanisms how high maternal lipid levels link to SPB risk remain poorly understood, studies have suggested that high lipids were associated with oxidative stress and systemic inflammation, and may also be related to placental dysfunction and microvascular injuries, which may result in SPB (Catov et al., 2008; Napoli and Lerman, 2001). Subsequently, we conducted a prospective nested case-control study in Shanxi Province, China, aiming to estimate the association of maternal serum Cu levels with SPB and to investigate the possible pathways of lipid metabolism.

2. Methods

2.1. Study design and participants

A nested case-control study was conducted in a prospective cohort of 4229 women from December 2009 to December 2013 in Shanxi Province, China. Women were recruited into the cohort if they met the following criteria: (Alebic-Juretic and Frkovic, 2005) prenatal examination at $\leq$22 gestational weeks, (Blencowe et al., 2012) at least 18 years old, (Carmichael et al., 2013) living in the local area for at least 1 year, and (Catov et al., 2008) agreed to provide signed informed consent. When the women attended their first prenatal examination, information about maternal social demographic characteristics was collected by trained healthcare workers using a structured questionnaire. Meanwhile, maternal blood was collected during 4–22 gestational weeks. The recruited women were prospectively followed up until delivery or pregnancy termination. Information about prenatal care, maternal and neonatal complications, and outcomes was recorded in a maternal health care booklet by healthcare workers. The study was approved by the Institutional Review Board of Peking University, and all participants provided written informed consent.

Among the cohort of 4229 women, women were excluded from the study if they withdrew (N = 216), were lost to follow-up (N = 83), terminated the pregnancy (N = 216), had iatrogenic preterm birth (N = 17), had multiple births (N = 34), were recruited at $<$ 4 or $>$ 22 gestational weeks (N = 18), did not have the information about fetal number (N = 16), last menstrual period (N = 3), delivery date (N = 116) or did not provide blood samples (N = 313). A total of 3197 women remained, including 198 with SPB (cases) and 2999 term births. Of the term births, we further excluded 204 births with low birth weight or small gestational age. Among the included term births, we randomly selected 396 women with term birth (controls) in a ratio of 1:2. Due to inadequate volume of some blood samples, a total of 147 cases and 381 controls were finally included in our nested case-control study (see selection diagram in Fig. 1). The population included in this study (N = 528) were similar to the overall population (N = 3197, see Table A.1). There were no mothers who gave birth before 22 gestational weeks. A total of 27 mothers were recruited during 21–22 gestational weeks and all of them gave birth after 34 gestational weeks.

2.2. Laboratory analyses

Maternal blood samples were collected by healthcare workers at the first prenatal visit. Two different tubes, i.e. red tube (Lot No. BD367814) for separating serum and purple tube (Lot No. BD367863) for separating plasma, were used to collect blood samples. Serum and plasma samples were then respectively transferred into screw-top vials, stored at $-80\,^{\circ}C$, and thawed prior before analysis. Serum concentrations of Cu were measured using inductively coupled plasma-mass spectrometry (ICP-MS; ELAN DRC II, PerkinElmer, USA). The measured Cu concentration in the standard serum samples (ClinChek® - Serum Control, Level II, RECIPE GmbH, Munich, Germany) was 1358 $\pm$ 44.7 ng/mL [median value $\pm$ standard deviation], which was consistent with the reference value, i.e., 1360 $\pm$ 204 ng/mL. The limit of detection was 0.03 ng/mL. Quantitative analysis was conducted at the Central Laboratory of Biological Elements in Peking University Health Science Center, and the protocol was qualified by the China Metrology Accreditation (CMA) system. Plasma TC and TG concentrations were measured using the oxidative method (INTEC (Xiamen) Technology Co., LTD, China) following the standard protocol. The optical density was quantified using an automatic biochemical analyzer (Olympus AU400, Japan).

2.3. Definitions

In this study, preterm birth was defined as live births with a

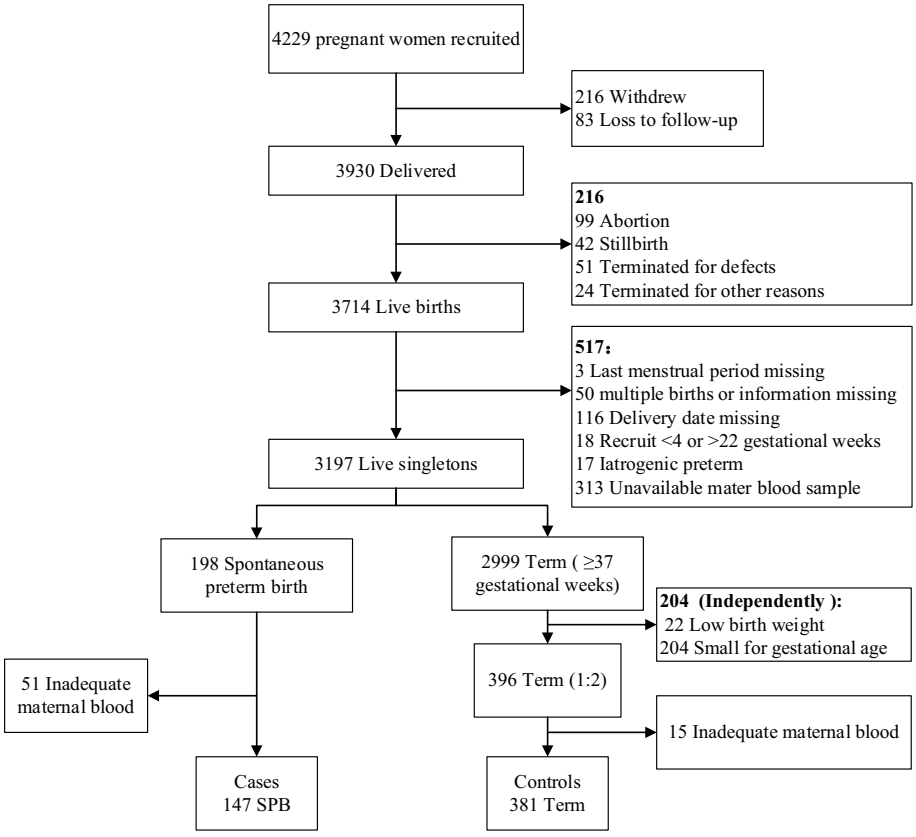

Fig. 1. Flow diagram of selection of cases and controls from the cohort.

gestational age of 20–36 weeks. Preterm birth was further categorized into two subtypes according to pregnancy complication. Iatrogenic preterm birth was defined as that caused by medical or obstetric complications, including pregnancy-induced hypertension, pre-eclampsia, chronic hypertension, diabetes, placenta praevia and placental abruption. The other cases were defined as SPB, including spontaneous preterm labor with intact membranes and PPROM. Controls were pregnant women who delivered at ≥37 gestational weeks. The maternal pre-pregnancy body mass index (BMI) was calculated based on the height and weight recorded in the questionnaire. Gestational age was calculated based on the first day of the maternal last menstrual period. The cutoff for first and second trimester was 13 weeks.

2.4. Statistical analyses

We compared maternal age, Han ethnicity, pre-pregnancy BMI, education, occupation, parity, spontaneous abortion history, folic acid use, medication use, pre-pregnancy passive smoking status, child gender, fasting status and sampling time between the case and control groups. Continuous and categorical variables were compared using *t*-tests and χ^2 tests, respectively. As Cu concentrations were not normally distributed, median and interquartile ranges (IQR) were used as descriptive parameters and the Mann-Whitney *U* test was used to compare the median between case and control groups. In the dose-response analysis, the concentrations of Cu, TC and TG were divided into four equal quartiles. An unconditional logistic regression analysis was used to estimate the adjusted odds ratios (OR) with 95% confidence intervals (CI) after adjusting for potential confounders. Because the regression standardized residuals by TC and TG approximately met the conditions of normal distribution and equal variance, we used linear regression to assess their relationships with serum Cu concentration. Because serum Cu concentration increased with gestational age (Izquierdo et al., 2007), we stratified all of the above-mentioned relationships by sampling time (first and second trimester). Moreover, categorical variables were treated as ordinal variables in the multivariate regression model to test the significance of the linear trend. Sensitivity analyses were also conducted to assess if the statistical results were stable enough. First, we conducted a sensitivity analysis by excluding participants who experienced placenta praevia ($N = 2$), placental abruption ($N = 3$), hypertension ($N = 9$) or diabetes ($N = 5$) in control group ($N = 12$), and who born small gestational age ($N = 29$) or low birth weight ($N = 5$) infants in case group ($N = 29$). Then, we conducted sensitivity analyses by excluding participants who had the history of previous preterm birth ($N = 8$) or stillbirth ($N = 8$), experienced post-term birth ($N = 24$) separately. Data were analyzed using SPSS for Windows software package (ver. 20.0; SPSS Inc., Chicago, Illinois, USA). A two-tailed p-value < 0.05 was considered statistically significant.

3. Results

In our study, the incidence of SPB was 5.3% (198/3714). A total of 528 women were included in the final analysis, including 147 cases and 381 controls; their characteristics were summarized in Table 1. Among them, 449 (85.2%) women were come from rural area, 73 (13.9%) women from urban or suburban area, and 5 women did not report the information. All women originated from the Han population and were nonsmokers. The participant characteristics, behaviors, child gender and the median gestational age of sampling time (13 weeks) were similar between the case and control groups. The median serum Cu concentration for all participants was 172 μg/dL (IQR, 136–198 μg/dL). Overall, the median serum Cu concentration of cases was significantly higher than that of controls (184 vs. 166 μg/dL, $p < 0.0001$). When stratified, the relationship was still significant for participants whose blood samples were collected during the first trimester (167 vs. 142 μg/dL, $p < 0.0001$) but not for those collected during the second trimester

Table 1
Characteristics of women who had SPB (cases) and women who delivered at ≥37 gestational weeks (controls) in a nested case-control study in Shanxi Province, China, 2009–2013.

Characteristics	No. of women	Cases (N = 147)	Controls (N = 381)	p
		No.[a] (%)	No.[a] (%)	
Age				
Mean (SD)		25.84 (4.77)	26.26 (4.46)	0.335
< 20	11	5 (3.4)	6 (1.6)	0.382
21–24	247	71 (48.6)	176 (46.2)	
25–29	171	47 (32.2)	124 (32.5)	
30–34	66	13 (8.9)	53 (13.9)	
≥35	32	10 (6.8)	22 (5.8)	
BMI (body mass index)				
Mean (SD)		22.23 (3.91)	22.72 (3.56)	0.175
< 18.5	55	19 (13.3)	36 (9.5)	0.246
18.5–24.9	342	96(67.1)	246 (65.3)	
≥25	123	28 (19.6)	95 (25.2)	
Education				
Primary or lower	35	12 (8.2)	23 (6.0)	0.488
Junior high	346	100 (68.0)	246 (64.6)	
High school	86	22 (15.0)	64 (16.8)	
College or higher	61	13 (8.8)	48 (12.6)	
Occupation				
Farmer	350	102 (69.4)	248 (65.3)	0.369
Other or unknown	177	45 (30.6)	132 (34.7)	
Parity				
Nulliparous	399	111 (75.5)	288 (75.6)	0.985
Multiparous	129	36 (24.5)	93 (24.4)	
Spontaneous abortion history				
Yes	68	19 (12.9)	49 (12.9)	0.984
No	460	128 (87.1)	332 (87.1)	
Folic acid use				
Yes	325	83 (58.0)	242 (64.7)	0.161
No	192	60 (42.0)	132 (35.3)	
Passive smoking				
Yes	246	68 (46.3)	178 (46.7)	0.924
No	282	79 (53.7)	203 (53.3)	
Medication use				
Yes	42	11 (7.5)	31 (8.1)	0.804
No	486	136 (92.5)	350 (91.9)	
Child gender				
Boy	285	82 (55.8)	203 (53.3)	0.605
Girl	243	65 (45.2)	178 (46.7)	
Fasting				0.597
Yes	159	48 (32.7)	111 (29.1)	
No	364	97 (66.0)	267 (70.1)	
Sampling time (weeks)				
Mean (SD)		13.22 (4.33)	13.07 (4.63)	0.737
First trimester	281	59 (50.3)	207 (54.3)	0.41
Second trimester	247	73 (49.7)	174 (45.7)	

SPB, spontaneous preterm birth; SD, standard deviation; CI, confidence interval.
[a] Values for some characteristics may not sum to the total number of preterm or term birth because of missing data.

Table 3
Association of maternal serum levels of Cu with SPB in a nested case-control study in Shanxi Province, China, 2009–2013.

Cu levels	Cases (N = 147) No. (%)	Controls (N = 381) No. (%)	Crude OR (95%CI)[a]	Adjusted OR (95%CI)[b]	Adjusted OR (95%CI)[c]
< 136.2	23 (15.7)	109 (28.6)	1	1	1
136.2–172.3	34 (23.1)	98 (25.7)	1.64 (0.91, 2.98)	1.87 (0.98, 3.56)	2.02 (1.07, 3.82)
172.4–198.7	40 (27.2)	92 (24.2)	2.06 (1.15, 3.69)	2.76 (1.38, 5.54)	3.10 (1.54, 6.22)
> 198.7	50 (34.0)	82 (21.5)	2.89 (1.63, 5.11)	3.91 (1.97, 7.73)	4.18 (2.11, 8.27)
p_{trend}			< 0.001	< 0.001	< 0.001

SPB, spontaneous preterm birth; Cu, copper, unit: μg/dL; OR, odds ratio; CI, confidence interval.
[a] Unconditional logistic regression with no adjustment.
[b] Unconditional logistic regression with adjustment for sampling time (continuous).
[c] Unconditional logistic regression with adjustment for sampling time (continuous), maternal age (continuous), BMI (continuous), education, occupation, parity, spontaneous abortion history, folic acid use, medication use, passive smoking, child gender and fasting.

(192 vs. 185 μg/dL, p = 0.085; see Table 2). Moreover, the overall median Cu concentration in the second trimester was significantly higher than that in the first trimester (188 vs. 148 μg/dL, $p < 0.0001$; see Table 2).

The association between high Cu concentration and the ORs of SPB showed a clear dose-response relationship ($p_{trend} < 0.0001$; Table 3). Compared to the lowest serum Cu level, the ORs of SPB increased to 2.02 (95% CI: 1.07, 3.82), 3.10 (1.54, 6.22) and 4.18 (2.11, 8.27) for the second, third and fourth quartiles respectively, after adjusting for maternal age, pre-pregnancy BMI, education, occupation, parity, spontaneous abortion history, folic acid use, medication use (≤22 gestational weeks), pre-pregnancy passive smoking status, child gender, fasting status and sampling time (see Table 3).

When the analysis was stratified by sampling time, the association was significant in the first trimester but not in the second trimester, regardless of whether the analysis was adjusted for potential confounders (see Fig. 2 and Table A.2).

The maternal plasma concentrations of TC and TG (μmol/L) increased as the serum concentration of Cu (μg/dL) increased. Estimates of the regression coefficient were 4.84 (95% CI: 3.08, 6.59; $p < 0.0001$) and 4.10 (95%CI: 2.45, 5.76; $p < 0.0001$) for TC and TG, respectively, after adjusting for potential confounders (Table 4). When the analysis was stratified by sampling time, the association was significant in first trimester regardless of whether the analysis was adjusted for potential confounders, but in second trimester the association between TG and Cu concentration was not significant when adjusted (Table A.3).

Table 2
Median Cu concentrations of women who had SPB (cases) and women who delivered at ≥37 gestational weeks (controls) in a nested case-control study in Shanxi Province, China, 2009–2013.

Groups	No. of women	Total population (N = 528) M (QR)	Cases (N = 147) M (QR)	Controls (N = 381) M (QR)	p[a]
Total	528	172 (136, 198)	184 (152,215)	166 (128, 193)	< 0.0001
First trimester	281	148 (117, 179)[b]	167 (135,204)	142 (112, 171)	< 0.0001
Second trimester	247	188 (170, 211)[b]	192 (174,224)	185 (168, 209)	0.085

SPB, spontaneous preterm birth; Cu, copper, unit: μg/dL; M, median; QR, quartile range.
[a] Mann–Whitney U test between cases and controls.
[b] $p < 0.0001$, Mann–Whitney U test between first trimester and second trimester in total population.

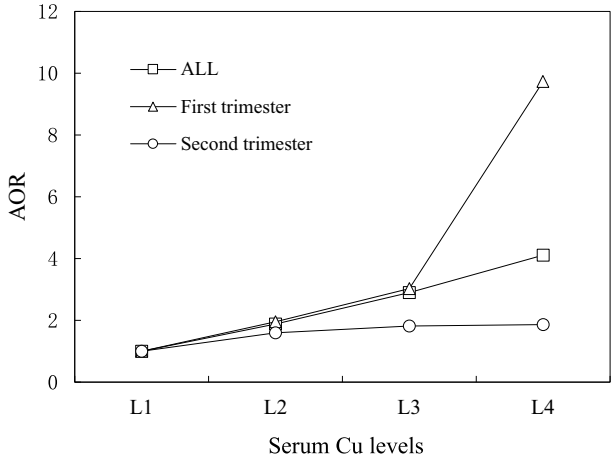

Fig. 2. Relationships of the adjusted odds ratio (OR) of maternal serum Cu with SPB in different trimesters. Serum Cu concentration was classified into four levels by quartiles of all subjects, i.e., < 1st quartile (L1), 1st–2nd quartile (L2), 2nd–3rd quartile (L3), > 3rd quartile (L4).

Table 4
Relationships of maternal plasma concentrations of total cholesterol and triglycerides with Cu concentration in a nested case-control study in Shanxi Province, China, 2009–2013.

Lipids	β (95%CI)	S_β	Standardized β	p
Total cholesterol				
Model 1[a]	8.62 (7.07, 10.18)	0.79	0.43	< 0.0001
Model 2[b]	4.84 (3.12, 6.56)	0.88	0.24	< 0.0001
Model 3[c]	4.84 (3.08, 6.59)	0.89	0.24	< 0.0001
Triglyceride				
Model 1[a]	7.35 (5.86, 8.85)	0.76	0.39	< 0.0001
Model 2[b]	4.42 (2.72, 6.11)	0.86	0.23	< 0.0001
Model 3[c]	4.10 (2.45, 5.76)	0.84	0.22	< 0.0001

Cu, copper, unit: μg/dL; Lipids, unit: μmol/L; OR, odds ratio; CI, confidence interval.

[a] Linear regression with no adjustment.

[b] Linear regression with adjustment for sampling time (continuous).

[c] Linear regression with adjustment for sampling time (continuous), maternal age (continuous), BMI (continuous), education, occupation, parity, spontaneous abortion history, folic acid use, medication use, passive smoking, child gender and fasting.

Compared to the lowest maternal plasma TC level, the ORs of SPB risk based on cholesterol increased to 3.16 (95%CI: 1.62, 6.16), 4.49 (95%CI: 2.30, 8.76) and 6.31 (95%CI: 3.02, 13.18) and the ORs based on TG increased to 2.05 (95%CI: 1.11, 3.77), 2.55 (95%CI: 1.36, 4.78) and 3.30 (95%CI: 1.68, 6.48) in the second, third, and fourth quartiles, respectively, after adjusting for potential confounders (Table 5). The results for TC and TG also exhibited clear dose-response relationships (TC: $p < 0.0001$; TG: $p = 0.0006$; Table 5). However, when stratified by sampling time, the relationships became more distinct (Table A.4). The relationships with SPB were still significant in the first trimester, but not in the second trimester, except for the association between the highest TC level and SPB (adjusted OR: 6.19, 95%CI: 1.25, 30.62; Table A.4).

To test the robustness of our results, we conducted a sensitivity analysis by excluding who experienced pregnancy-induced hypertension, preeclampsia, chronic hypertension, diabetes, placenta praevia and placental abruption in control group ($N = 12$), and who born small gestational age or low birth weight infants in case group ($N = 29$) (Tables A.5–A.7). The results were similar to primary analyses. We also conducted sensitivity analyses by excluding participants who consumed alcohol during pregnancy ($N = 4$), had the history of previous preterm birth ($N = 8$) or stillbirth ($N = 8$), experienced post-term birth ($N = 24$) respectively, and the results did not change significantly.

Table 5
Associations of plasma levels of total cholesterol and triglycerides with SPB in a nested case-control study in Shanxi Province, China, 2009–2013.

Lipids levels	Cases ($N = 147$) No. (%)	Controls ($N = 381$) No. (%)	Crude OR (95%CI)[a]	Adjusted OR (95%CI)[b]
Total cholesterol				
< 3.61	17 (11.5)	115 (30.2)	1	1
3.61–4.23	37 (25.2)	95 (24.9)	2.64 (1.40, 4.97)	3.16 (1.62, 6.16)
4.24–4.84	46 (31.3)	89 (23.4)	3.50 (1.88, 6.51)	4.49 (2.30, 8.76)
> 4.84	47 (32.0)	82 (21.5)	3.88 (2.08, 7.23)	6.31 (3.02, 13.18)
P_{trend}			< 0.0001	< 0.0001
Triglyceride				
< 1.10	24 (16.3)	109 (28.6)	1	1
1.10–1.54	38 (25.9)	92 (24.1)	1.88 (1.05, 3.36)	2.05 (1.11, 3.77)
1.55–2.10	41 (27.9)	93 (24.4)	2.00 (1.13, 3.56)	2.55 (1.36, 4.78)
> 2.10	44 (29.9)	87 (22.9)	2.30 (1.30, 4.07)	3.30 (1.68, 6.48)
P_{trend}			0.0061	0.0006

SPB, spontaneous preterm birth; Lipids, unit: μmol/L; OR, odds ratio; CI, confidence interval.

[a] Unconditional logistic regression with no adjustment.

[b] Unconditional logistic regression with adjustment for sampling time (continuous), maternal age (continuous), BMI (continuous), education, occupation, parity, spontaneous abortion history, folic acid use, medication use, passive smoking, child gender and fasting.

4. Discussion

In this prospective nested case-control study, we found that concentrations of Cu, TC and TG were positively associated with an increased risk of SPB in a dose-dependent manner in the first trimester but not in the second trimester. Moreover, the concentrations of TC and TG increased with increasing Cu concentrations. These results support the hypothesis that maternal Cu level is associated with SPB risk due to increases in the concentrations of TC and TG in the first, but not the second trimester.

In this study, serum Cu concentrations in the first and second trimesters were similar to those reported by the China Nutrition and Health Survey 2010–2012 of pregnant women [first trimester (≤11 weeks): 153 μg/dL, IQR, 124–195; second (12–27 weeks): 197 μg/dL, IQR, 169–223] (Liu et al., 2016). However, serum Cu concentrations observed in this study were higher than those reported in a Turkish study (Kilinc et al., 2010) and higher than Cu concentrations in umbilical cord serum in a Chinese cohort study (Li et al., 2018). Our observed concentrations were lower than plasma concentrations reported in an Australian (Wilson et al., 2018) and a Croatian study (Alebic-Juretic and Frkovic, 2005). These differences may be caused by differences in sample type, country, and sampling time. However, as suggested by previous studies, Cu concentrations in serum are a reliable indicator of the Cu levels in both depleted and replete populations, unlike plasma and other methods (Harvey et al., 2009). Therefore, the serum Cu concentrations observed in our study can be considered representative of the maternal Cu level in China at some scale.

Several previous studies have estimated the association between Cu level and preterm birth, but most of these studies have been conducted post delivery and did not separate SPB from iatrogenic preterm birth. As far as we know, only one cohort study examined the relationship between maternal Cu level and SPB in the first trimester (Wilson et al., 2018). These authors reported that, compared to women with high Cu (3rd tertile), women with lower Cu had a decreased risk for SPB [2nd tertile: adjusted RR = 0.52 (0.28, 0.98); 1st tertile: adjusted RR = 0.62 (0.34, 1.15)] (Wilson et al., 2018). In other words, the study suggested that compared to women with lower copper level women with high copper had increased risk, which is consistent with our findings in the

first trimester. However, in the cohort study, Cu concentrations were measured in plasma and not serum, as in our study (Wilson et al., 2018). Several previous studies have reported that Cu deficiency in newborn serum, umbilical cord blood (serum and plasma) and placenta were correlated with preterm birth (Li et al., 2018; Perveen et al., 2002; Schulpis et al., 2004; Zadrożna et al., 2009). In contrast, other studies have not found an association between maternal plasma Cu intake and preterm birth (Alebic-Juretic and Frkovic, 2005). These discrepancies may be due to differences in population, study design, sample type or sampling time. Most previous studies have been case-control studies and Cu concentrations were determined in samples collected post delivery from newborn blood, umbilical cord blood, or placenta, resulting in differences in the sampling time between preterm birth (< 37 weeks) and term birth (≥37 weeks). However, Cu concentrations in pregnancy increase with gestational age (Izquierdo et al., 2007). Compared to the term group, the Cu deficiency in the preterm group may have resulted from the smaller gestational age of sampling time in the preterm group and, to some extent, even the high Cu level in the preterm group may have been covered by the difference in sampling time. Moreover, general case-control studies are limited in their capacity to identify causal relationships because of the limitation of distinguishing the time order. In our prospective nested case-control study, the sampling time occurred before delivery (4–22 gestational weeks) and was similar (13 weeks) for the case and control groups; therefore, our results may be more likely to represent a causal relationship between high maternal Cu level and SPB risk.

Likewise, we found that plasma concentrations of TC and TG increased with increasing serum Cu, which was consistent with most previous animal and epidemiology studies of other populations (Galhardi et al., 2004; Shoji et al., 2017; Wells et al., 2014). Previous animal studies reported that the increased lipid profile induced by Cu is associated with oxidative stress, since high Cu intake increased lipid hydroperoxide concentrations and reduced the superoxide dismutase and glutathione peroxidase activities (Galhardi et al., 2004). Furthermore, we found that increased plasma concentrations of TC and TG were positively associated with an increased risk of SPB. This finding is consistent with a series of studies by Catov et al., who consistently demonstrated that dyslipidemia in the first trimester was associated with more than twice the OR of SPB (Catov et al., 2008; Catov et al., 2007a, b). Moreover, a recent meta-analysis reported that elevated maternal lipids were associated with a higher risk of SPB (OR = 1.67, 95% CI: 1.05, 2.68, $p = 0.031$), but not of iatrogenic preterm birth (OR = 1.11, 95% CI: 0.64, 1.91, $p = 0.715$) (Jiang et al., 2017). The mechanisms of how increased maternal lipid levels link to SPB remain poorly understood. However, previous studies have suggested that high lipids levels are associated with oxidative stress and systemic inflammation and may also be related to placental dysfunction or microvascular injuries that lead to SPB (Catov et al., 2008; Kelly et al., 2009). Our findings suggest that high maternal Cu level may increase the risk of SPB, by potentially increasing plasma concentrations of TC and TG.

Several limitations should be considered when interpreting our results. First, all participants in this study were recruited in Shanxi province of China and of Han ethnicity, thus extrapolation of our results to other populations with different demographic characteristics or in other areas should be careful. Second, other internal biomarkers of lipid metalation between Cu and SPB were not measured and we did not know if TC/TG is a confounder or a mediator, which limits the interpretation of potential pathways. Third, the information on maternal drug abuse during pregnancy were not collected. Finally, the sample size was relatively small for some of the subgroups when the analysis was stratified.

However, our study has several advantages compared to previous studies. First, all of the cases and controls included in our study came from the same population of the cohort, and the baseline characteristics were similar among them, such as ethnicity, education level and occupation. Therefore, the homogeneity of genetic and exposure backgrounds helped to reduce the residual confounding. Second, this study was nested in a prospective cohort, which allowed us to record exposure and outcome data prospectively and to minimize the potential for selection and recall bias. In our study, all of the concentrations were determined using maternal blood collected during 4–22 gestational weeks, which allows us estimate a possible causal relationship between maternal Cu and SPB and to provide predictive and preventive values for SPB in the first trimester. Third, the sampling time for the cases and controls in our study were similar (i.e., 13 weeks), which can minimize the effects of gestational age on increased concentrations of Cu, TC and TG during pregnancy. Fourth, we only included the SPB cases rather than all preterm cases (iatrogenic preterm birth and SPB), so we were able to examine the relationships with SPB more precisely. Finally, the sample size in our study was larger than that in most previous studies.

5. Conclusion

Our study provides unique insight into the effects of maternal Cu level on SPB development, as well as the potential mechanisms in terms of lipid metabolism. Our results suggest that high maternal Cu intake in the first trimester may increase the risk of SPB by potentially increasing the concentrations of TC and TG.

Conflict of interest

The authors declare they have no actual or potential competing financial interests.

Acknowledgments

We appreciate the contributions of all volunteers in this study. This work was supported by the National Natural Science Foundation of China [Grant No. 81673177 and No. 41771527].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.11.009.

References

Alebic-Juretic, A., Frkovic, A., 2005. Plasma copper concentrations in pathological pregnancies. J. Trace Elem. Med. Biol. 19, 191–194.
Blencowe, H., Cousens, S., Oestergaard, M.Z., Chou, D., Moller, A., Narwal, R., et al., 2012. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 379, 2162–2172.
Carmichael, S., Yang, W., Shaw, G., 2013. Maternal dietary nutrient intake and risk of preterm delivery. Am. J. Perinatol. 30, 579–588.
Catov, J.M., Bodnar, L.M., Ness, R.B., Barron, S.J., Roberts, J.M., 2007a. Inflammation and dyslipidemia related to risk of spontaneous preterm birth. Am. J. Epidemiol. 166, 1312–1319.
Catov, J.M., Bodnar, L.M., Kip, K.E., Hubel, C., Ness, R.B., Harger, G., et al., 2007b. Early pregnancy lipid concentrations and spontaneous preterm birth. Am. J. Obstet. Gynecol. 197, 610–611.
Catov, J.M., Bodnar, L.M., Hackney, D., Roberts, J.M., Simhan, H.N., 2008. Activation of the fibrinolytic cascade early in pregnancy among women with spontaneous preterm birth. Obstet. Gynecol. 112, 1116–1122.
Dekker, G.A., Lee, S.Y., North, R.A., McCowan, L.M., Simpson, N.A., Roberts, C.T., 2012. Risk factors for preterm birth in an international prospective cohort of nulliparous women. PLoS One 7, e39154.
Galhardi, C.M., Diniz, Y.S., Faine, L.A., Rodrigues, H.G., Burneiko, R.C.M., Ribas, B.O., et al., 2004. Toxicity of copper intake: lipid profile, oxidative stress and susceptibility to renal dysfunction. Food Chem. Toxicol. 42, 2053–2060.
Goldenberg, R.L., Culhane, J.F., Iams, J.D., Romero, R., 2008. Epidemiology and causes of preterm birth. Lancet 371, 75–84.
Harvey, L.J., Ashton, K., Hooper, L., Casgrain, A., Fairweather-Tait, S.J., 2009. Methods of assessment of copper status in humans: a systematic review. Am. J. Clin. Nutr. 89, 2009S–2024S.
Izquierdo, A.S., Castanon, S.G., Ruata, M.L., Aragues, E.F., Terraz, P.B., Irazabal, Y.G., et al., 2007. Updating of normal levels of copper, zinc and selenium in serum of pregnant women. J. Trace Elem. Med. Biol. 21 (Suppl. 1), 49–52.

Jiang, S., Jiang, J., Xu, H., Wang, S., Liu, Z., Li, M., et al., 2017. Maternal dyslipidemia during pregnancy may increase the risk of preterm birth: a meta-analysis. Taiwan. J. Obstet. Gynecol. 56, 9–15.
Kelly, R., Holzman, C., Senagore, P., Wang, J., Tian, Y., Rahbar, M.H., et al., 2009. Placental vascular pathology findings and pathways to preterm delivery. Am. J. Epidemiol. 170, 148–158.
Kilinc, M., Coskun, A., Bilge, F., Imrek, S.S., Atli, Y., 2010. Serum reference levels of selenium, zinc and copper in healthy pregnant women at a prenatal screening program in southeastern Mediterranean region of Turkey. J. Trace Elem. Med. Biol. 24, 152–156.
Li, Z., Liang, C., Huang, K., Yan, S., Tao, R., Sheng, J., et al., 2018. Umbilical serum copper status and neonatal birth outcomes: a prospective cohort study. Biol. Trace Elem. Res. 183, 200–208.
Liu, L., Johnson, H.L., Cousens, S., Perin, J., Scott, S., Lawn, J.E., et al., 2012. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 379, 2151–2161.
Liu, X., Piao, J., Zhang, Y., Li, M., Li, W., Yang, L., et al., 2016. Serum copper status in school-age children and pregnant women in China nutrition and health survey 2010–2012. Biol. Trace Elem. Res. 173, 268–274.
Mudd, L.M., Holzman, C.B., Catov, J.M., Senagore, P.K., Evans, R.W., 2012. Maternal lipids at mid-pregnancy and the risk of preterm delivery. Acta Obstet. Gynecol. Scand. 91, 726–735.
Napoli, C., Lerman, L.O., 2001. Involvement of oxidation-sensitive mechanisms in the cardiovascular effects of hypercholesterolemia. Mayo Clin. Proc. 76 (6), 619–631.
Perveen, S., Altaf, W., Vohra, N., Bautista, M.L., Harper, R.G., Wapnir, R.A., 2002. Effect of gestational age on cord blood plasma copper, zinc, magnesium and albumin. Early Hum. Dev. 69, 15–23.
Schulpis, K.H., Karakonstantakis, T., Gavrili, S., Costalos, C., Roma, E., Papassotiriou, I., 2004. Serum copper is decreased in premature newborns and increased in newborns with hemolytic jaundice. Clin. Chem. 50, 1253–1256.
Shoji, H., Ikeda, N., Kojima, C., Kitamura, T., Suganuma, H., Hisata, K., et al., 2017. Relationship between copper and lipids and atherogenic indices soon after birth in Japanese preterm infants of 32–35 weeks. J. Dev. Orig. Health Dis. 8, 256–260.
Song, X., Li, B., Li, Z., Wang, J., Zhang, D., 2017. High serum copper level is associated with an increased risk of preeclampsia in Asians: a meta-analysis. Nutr. Res. 39, 14–24.
Wells, E.M., Navas-Acien, A., Apelberg, B.J., Herbstman, J.B., Jarrett, J.M., Lin, Y.H., et al., 2014. Association of selenium and copper with lipids in umbilical cord blood. J. Dev. Orig. Health Dis. 5, 281–287.
Wilson, R.L., Bianco-Miotto, T., Leemaqz, S.Y., Grzeskowiak, L.E., Dekker, G.A., Roberts, C.T., 2018. Early pregnancy maternal trace mineral status and the association with adverse pregnancy outcome in a cohort of Australian women. J. Trace Elem. Med. Biol. 46, 103–109.
Zadrożna, M., Gawlik, M., Nowak, B., Marcinek, A., Mrowiec, H., Walas, S., et al., 2009. Antioxidants activities and concentration of selenium, zinc and copper in preterm and IUGR human placentas. J. Trace Elem. Med. Biol. 23, 144–148.
Zou, L., Wang, X., Ruan, Y., Li, G., Chen, Y., Zhang, W., 2014. Preterm birth and neonatal mortality in China in 2011. Int. J. Gynaecol. Obstet. 127, 243–247.