Clinical Nutrition 38 (2019) 2922–2927

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

# Association between plasma concentration of copper and gestational diabetes mellitus

Peiyun Li [a, b], Jiawei Yin [a, b], Yalun Zhu [a, b], Shuzhen Li [a, b], Sijing Chen [a, b], Taoping Sun [a, b], Zhilei Shan [a, b, c], Jiawei Wang [a, b], Qianqian Shang [a, b], Xiaoqin Li [a, b], Wei Yang [a, b, **], Liegang Liu [a, b, *]

[a] Department of Nutrition and Food Hygiene, Hubei Key Laboratory of Food Nutrition and Safety, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, PR China
[b] Ministry of Education Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, PR China
[c] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA

ARTICLE INFO

*Article history:*
Received 1 October 2018
Accepted 26 December 2018

*Keywords:*
Copper
Gestational diabetes mellitus
Case-control study
Pregnancy

SUMMARY

*Background & aims:* Emerging findings have raised concerns about significant associations between excessive copper (Cu) and abnormal glucose metabolism. Nevertheless, related researches on the relationship of Cu concentration and gestational diabetes mellitus (GDM) are limited. The objective of this study was to determine whether plasma Cu concentration is associated with GDM.

*Methods:* A case–control study of 248 cases of GDM and 248 age-, parity- and gestational age-matched controls was conducted in Wuhan, China between August 2012 and April 2015. Fasting blood samples of participants were collected at the time of GDM screening (≥24 weeks of gestation). Plasma Cu concentrations were detected by inductively coupled plasma mass spectrometry. The strength of the association of plasma Cu with GDM odds was evaluated by odds ratios (ORs) with 95% confidence intervals (CIs) from conditional logistic regression. Partial Spearman or Pearson correlation coefficients were calculated to estimate the interrelationship between plasma Cu and the risk factors of GDM.

*Results:* Plasma Cu concentrations in the GDM group (mean ± SD: 1960.24 ± 391.98 μg/L) were higher than in the control group (mean ± SD: 1842.43 ± 387.09 μg/L) ($P = 0.001$). After adjustment for possible confounders, the ORs (95% CIs) of GDM across increasing quartiles of plasma Cu levels were 1.00 (referent), 1.79 (0.90–3.55), 2.72 (1.35–5.48) and 2.91 (1.48–5.75), respectively; the OR (95% CI) of GDM was 1.33 (1.06–1.67) for each standard deviation increment of plasma Cu. Moreover, Cu concentrations were positively associated with fasting plasma glucose, 1-h post-glucose load and 2-h post-glucose load (all $P < 0.05$).

*Conclusions:* The present study indicated a significantly increased odds of GDM in association with higher concentrations of plasma Cu. Prospective cohort studies in other populations are needed to confirm our findings.



*Abbreviations:* AGE, advanced glycation end-product; BMI, body mass index; CI, confidence interval; Cu, copper; FPG, fasting plasma glucose; FPI, fasting plasma insulin; GDM, gestational diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment; IQR, interquartile range; IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; OR, odds ratio; ROS, reactive oxygen species; SOD, superoxide dismutase; SD, standard deviation; TC, total cholesterol; TG, triglycerides.

* Corresponding author. Department of Nutrition and Food Hygiene, Hubei Key Laboratory of Food Nutrition and Safety, and Ministry of Education Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan 430030, China. Fax: +86 27 83650522.

** Corresponding author. Department of Nutrition and Food Hygiene, Hubei Key Laboratory of Food Nutrition and Safety, and Ministry of Education Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan 430030, China. Fax: +86 27 83650522.

*E-mail addresses:* yw8278@hotmail.com (W. Yang), lgliu@mails.tjmu.edu.cn (L. Liu).

https://doi.org/10.1016/j.clnu.2018.12.032

## 1. Introduction

Gestational diabetes mellitus (GDM), defined as glucose intolerance with onset or first recognition during pregnancy, is present in about 14% of all pregnancies worldwide in 2017 [1], making it one of the most common complications of pregnancy. GDM is associated with considerable risks to both the mother and developing fetus. Hyperglycemia increases the risk of a series of adverse obstetric and neonatal complications including caesarean delivery, preterm birth, macrosomia and neonatal metabolic disturbances [2]. Women who have GDM are more likely to develop GDM in subsequent pregnancies and about half of them will develop type 2 diabetes within five to ten years after delivery [3]. Babies born to mothers with GDM also have an increased lifetime risk of obesity and developing type 2 diabetes [4]. Although GDM is a disease of the pancreatic β cells producing insufficient insulin to meet the increased requirements of late pregnancy, the underlying causes of β-cell dysfunction haven't been well understood [5]. Thus, evaluating modifiable pathophysiologic changes associated with GDM could impact both maternal and neonatal outcomes.

As a redox active metal, copper (Cu) is an essential nutrient for the normal human physiology and function of over 30 proteins in metabolism, including superoxide dismutase (SOD), ceruloplasmin, cytochrome *c* oxidase and dopamine-β-hydroxylase [6]. The main source of Cu for humans is food including seafood, organ meats, grains and legumes while drinking water encompasses 20–25% of the dietary Cu ingested [7]. Cu misbalance could produce either functional or structural abnormalities [8,9]. Recent years, emerging evidences from both human and animal studies have raised concerns about significant associations between excessive Cu and abnormal glucose metabolism. A meta-analysis involving 15 eligible studies indicated that patients with diabetes carried higher levels of plasma/serum Cu than healthy individuals [10], and Lin et al. [11] and Naka et al. [12] found a positive correlation between Cu and HbA1c. In diabetic db/db mice, Tanaka and colleagues found that treatment with a Cu chelating agent reduced reactive oxygen species (ROS) levels, and ameliorated insulin resistance and glucose intolerance [13]. Furthermore, the role of Cu in ROS generation, oxidation of glutamic acid decarboxylase, and β cell apoptosis were demonstrated in vitro studies [14,15]. Therefore, maintaining Cu homeostasis seems to be imperative for the prevention and treatment of abnormal glucose metabolism. According to previous studies, serum/plasma concentrations of Cu were higher in pregnant women than in the general population [16]. Normal pregnancies are always accompanied by some evident endocrine changes that give rise to the fact that pregnant women are particularly vulnerable to Cu misbalance [17,18]. However, up to date, related researches on the relationship of Cu concentration and GDM are limited, and the results are inconsistent [19–22]. In addition, the small sample size and unmatched groups of previous studies could contribute to the conflicting association between Cu and GDM.

Therefore, we conducted a matched case–control study in a Chinese population to test the hypothesis that plasma Cu was positively associated with GDM and risk factors of GDM.

## 2. Materials and methods

### 2.1. Study population and design

The case–control study was conducted in Wuhan, China between August 2012 and April 2015. Participants were recruited from pregnant women screening for GDM at the outpatient clinics of the Department of Endocrinology, Tongji Hospital. The inclusion criteria of participants were: age ≥20 years, gestational age at GDM screening ≥24 weeks. We excluded subjects with a history of diagnosis of diabetes or GDM, those received pharmacologic treatment known to affect glucose metabolism, and those with systemic diseases and multiple pregnancies. Maternal fasting blood samples were collected in heparin anticoagulant tubes and centrifuged at 3000 rpm for 5 min. Then, the plasma was separated and stored at −80 °C until analysis. The study was approved by the Ethics Committee of Tongji Medical College. Written informed consent was obtained from all the participants.

### 2.2. GDM case definition and control selection

GDM was diagnosed by a 75 g oral glucose tolerance test (OGTT) in accordance with the American Diabetes Association guidelines [23]. Normal results were defined as: level of blood glucose <5.1 mmol/L (92 mg/dL) at baseline, < 10.0 mmol/L (180 mg/dL) at 1 h, < 8.5 mmol/L (153 mg/dL) at 2 h. A diagnosis of GDM was given if one or more tests had abnormal value. Control subjects were chosen randomly from women who passed the OGTT and 1:1 matched to GDM cases according to age (±2 years), gestational age at blood drawing (±2 weeks), and parity [24].

### 2.3. Measurement of plasma Cu concentrations

Plasma concentrations of Cu were detected by inductively coupled plasma mass spectrometry (Agilent 7700 Series ICP-MS; Agilent Technologies, USA) in the Ministry of Education Key Laboratory of Environment and Health at the School of Public Health at Tongji Medical College of Huazhong University of Science and Technology. The case–control status of study participants was not disclosed to the testing procedure. Prior to analysis, all samples were thawed, mixed thoroughly by vortex. Digestive solution was composed of 2% (w/w) $HNO_3$, 0.04% (w/w) Triton X-100 and 1% (w/w) Butan-1-ol. Then, samples (40 μL plasma) were diluted with ultrapure water and digestive solution at a ratio of 1:19:20. The detection limit of Cu was 0.0097 μg/L, and the concentration of the lowest standard solution (0.02 μg/L) was considered as the limit of quantitation. Calibration curve ranged from 0.02 μg/L to 100 μg/L was used to determine the concentration of plasma Cu. In addition, 1 mg/L internal standard composed of germanium in nitric acid was pumped into the instrument with the calibration standards or preprocessed samples at a rate of 0.10 m/s. This is done to correct for the loss of analyte during sample inlet. For quality assurance, the certified reference agents ClinChek No. 8883 and No. 8884 human plasma controls for trace elements were analyzed in every 20 samples. The concentrations of Cu were 697.6 ± 49.9 μg/L (certified: 692.0 ± 138.0 μg/L) and 1284.7 ± 33.4 μg/L (certified: 1220.0 ± 244.0 μg/L) for No. 8883 and No. 8884, respectively. The intra-assay and inter-assay coefficients of variation of plasma Cu were <5%.

### 2.4. Assessment of covariates

Data on demographics, socioeconomic status, lifestyle and health information were collected by trained investigators through face-to-face interviews with structured questionnaires. Prepregnancy body mass index (BMI) was calculated as self-reported prepregnancy weight divided by the square of height ($kg/m^2$). Fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assessed with the use of commercial assay kits (Biosino Bio-Technology and Science Inc., Beijing, China). Assay for fasting plasma insulin (FPI) was performed by enzyme linked immunosorbent assay kits (Mercodia Company, Sweden). The estimate of insulin resistance

(IR) by homeostasis model assessment (HOMA) score was calculated using the following formulas: HOMA-IR = FPG (mmol/L) × FPI (mU/L)/22.5.

### 2.5. Statistical methods

Variables were summarized as means ± standard deviations (SDs) (normal distributed) or medians with interquartile ranges (IQRs) (non-normal distributed) if continuous, and as percentage if categorical. Comparison between groups were performed with Student's t test or Mann–Whitney U test for continuous variables, and chi-square test for categorical variables. Subjects were divided into quartiles based on Cu concentrations among the controls, and the strength of the association of plasma Cu with GDM odds was evaluated by odds ratios (ORs) with 95% confidence intervals (CIs) from conditional logistic regression, with adjustment for age, prepregnancy BMI, gestational age, parity, family history of diabetes, drinking and smoking status [25]. The median value of each quartile of Cu was considered as a continuous variable in the logistic regression models to test for a linear trend. Associations of plasma Cu levels with FPG, 1-h post-glucose load, 2-h post-glucose load, FPI, HOMA-IR, TC, TG, LDL-C and HDL-C were estimated by Spearman or Pearson correlation coefficients according to the distribution of relevant variables. After adjusting for the covariates aforementioned, partial correlation coefficients were calculated. Statistical analyses were performed using SPSS 20.0 software package (SPSS Inc.). A two-tailed $P$ value < 0.05 was considered statistically significant.

## 3. Results

Among the 651 eligible women who agreed to attend the study, 3 had missing information on age or parity. After exclusions, 312 women with GDM and 336 healthy pregnant women were eligible for case–control matching according to the methods. Finally, 248 GDM cases and 248 matched controls were selected by SPSS software for further analyses. Characteristics of the participants are presented in Table 1. No relevant differences were showed in age, parity and gestational age between groups. Compared to controls, women with GDM were more likely to have a family history of diabetes, higher prepregnancy BMI, higher concentrations of FPG, 1-h OGTT, 2-h OGTT, FPI, TG and HOMA-IR index. Additionally, women who developed GDM displayed significantly increased Cu concentrations compared with control subjects (mean ± SD: 1960.24 ± 391.98 μg/L in GDM group, 1842.43 ± 387.09 μg/L in control group, $P = 0.001$).

Table 2 displays the results of crude and adjusted logistic regression analyses between plasma Cu concentration and GDM odds. The ORs (95% CIs) of GDM across increasing quartiles of plasma Cu levels were 1.00 (referent), 1.60 (0.89–2.85), 2.44 (1.35–4.39) and 3.01 (1.68–5.38), respectively. The results remained robust after adjusting for age, prepregnancy BMI, gestational age at blood drawing, family history of diabetes, drinking and smoking status, which indicated that women in the highest quartile had 2.91 times the odds (95% CI 1.48–5.75) of developing GDM compared to the reference quartile (lowest quartile). Furthermore, the ORs (95% CI) of GDM for each SD increment of plasma Cu was 1.33 (1.06–1.67).

Significant positive correlations were identified between plasma Cu and FPG, 1-h OGTT, 2-h OGTT, FPI, HOMA-IR and TG concentrations (all $P < 0.05$) (Table 3). After adjustment for covariates aforementioned, Cu concentrations remained positively associated with FPG ($P = 0.001$, $r = 0.147$), 1-h OGTT (P = 0.043, r = 0.092) and 2-h OGTT ($P = 0.002$, $r = 0.141$).

## 4. Discussion

In this case–control study, we found that plasma Cu concentrations were significantly and positively associated with GDM odds, independent of the established risk factors for GDM [26]. Moreover, plasma Cu was positively correlated with FPG, 1-h post-glucose load and 2-h post-glucose load.

We for the first time examined plasma Cu concentrations in relation to odds of GDM in a relatively large sample size. Previous studies only focused on the difference of Cu concentrations between GDM case and control groups, without further exploration of the relation between Cu and GDM risk due to the small sample size [19–22]. For instance, in the case–control study conducted by Wang et al., in 2002 [19], Cu concentrations in GDM group (n = 46) were significantly higher than those in healthy pregnant group (n = 90), which was consistent with our results. However, a couple of studies assessing the association between essential trace elements and GDM indicated that no statistically significant differences were found in serum Cu concentrations between healthy pregnant women and women with GDM [20–22]. In addition, Loven et al. found that no significant difference in serum concentrations of ceruloplasmin, a blue colored protein that binds up to 95% of circulating Cu [27], between GDM case and control groups, while the activity of ceruloplasmin in women with GDM was slightly higher [20]. Considering that the sample sizes of the scarce studies were limited and few confounding factors were adjusted in the analyses, the results should be interpreted with caution. On the other hand, our findings regarding the positive relationship between plasma Cu levels and GDM odds are in agreement with previous studies on type 2 diabetes among non-pregnant populations. Recently, a meta-analysis involving a total of 1079 diabetic patients and 561 healthy controls identified that patients with diabetes carried higher levels of Cu than healthy individuals. Stratification analyses also revealed similar results [10]. Besides, a prospective cohort study encompassing 16160 Japanese participants found that dietary intake of Cu, which was obtained by a validated food frequency questionnaire, was positively associated with risk of type 2 diabetes, and people in the highest quartile of Cu intake had 1.55-fold risk (95% CI 1.13–2.02) of developing diabetes compared with those in the lowest quartile [28]. In view of the study design and large sample sizes of these two studies, the positive results lent convincing support to the hypothesis that Cu was involved in the pathogenesis of abnormal glucose metabolism.

In our study, the mean concentrations of plasma Cu in women with or without GDM were 1960.24 μg/L and 1842.43 μg/L, respectively, which were higher than in the non-pregnant population in other studies [29,30]. Previous studies conducted in different countries have also found that serum Cu levels were higher in pregnant women than in non-pregnant healthy subjects [16,31,32]. These differences may be attributed to several factors. The increased need for maternal Cu-dependent enzymes such as SOD and cytochrome *c* oxidase might lead to the rise of Cu absorption during pregnancy [16]. In addition, dietary habits often change a lot after pregnancy. The pregnant women are prone to consume more food rich in Cu such as animal foods [33]. Moreover, Arredondo et al. found that concentrations of circulating estrogens in healthy adults could influence a series of indicators customarily used to assess nutritional status of Cu like serum Cu and ceruloplasmin. Results of the cellular experiments also supported the in vivo data [34]. Thus, hormonal changes during pregnancy may be part of the explanation for higher circulating Cu concentrations observed in gestational women in comparison to non-pregnancy subjects. However, on the other hand, plasma Cu levels in this population were similar to those in pregnant women reported in

**Table 1**
Characteristics of the GDM and control groups.[a]

| Characteristics | GDM (N = 248) | Non-GDM (N = 248) | *P* |
|---|---|---|---|
| Age, y | 30.03 ± 3.73 | 29.41 ± 3.81 | 0.067 |
| Parity, n (%) | | | 1.000 |
| 1 | 201 (81.05) | 201 (81.05) | |
| 2 | 45 (18.15) | 45 (18.15) | |
| ≥3 | 2 (0.80) | 2 (0.80) | |
| Gestational age at blood drawing, wk | 28.00 (26.00–30.00) | 28.00 (26.00–30.00) | 0.985 |
| Prepregnancy BMI, kg/m$^2$ | 22.18 ± 3.09 | 20.68 ± 2.71 | <0.001 |
| Family history of diabetes, n (%) | 63 (25.40) | 35 (14.11) | 0.001 |
| Alcohol consumers, n (%) | 12 (4.84) | 10 (4.03) | 0.663 |
| Smokers, n (%) | 4 (1.61) | 5 (2.02) | 0.737 |
| Fasting plasma glucose, mmol/L | 5.23 (5.06–5.47) | 4.72 (4.60–4.93) | <0.001 |
| OGTT-1h, mmol/L | 9.69 (8.59–10.92) | 7.42 (6.51–8.43) | <0.001 |
| OGTT-2h, mmol/L | 8.62 (7.49–9.35) | 6.88 (6.16–7.64) | <0.001 |
| Fasting plasma insulin, μU/mL | 10.40 (7.83–14.07) | 8.08 (6.19–10.49) | <0.001 |
| HOMA-IR | 2.47 (1.75–3.33) | 1.68 (1.27–2.28) | <0.001 |
| Total cholesterol, mmol/L | 5.49 (4.79–6.34) | 5.49 (4.78–6.23) | 0.606 |
| Triglycerides, mmol/L | 2.83 (2.33–3.69) | 2.51 (2.02–3.43) | 0.002 |
| LDL cholesterol, mmol/L | 3.27 (2.51–3.98) | 3.16 (2.62–3.85) | 0.796 |
| HDL cholesterol, mmol/L | 1.36 (1.17–1.57) | 1.39 (1.14–1.63) | 0.648 |
| Cu, μg/L | 1960.24 ± 391.98 | 1842.43 ± 387.09 | 0.001 |

Abbreviations: BMI, body mass index; Cu, copper; HDL, high density lipoprotein; GDM, geatational diabetes mellitus; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low density lipoprotein; OGTT-1h, 1-h post-glucose load; OGTT-2h, 2-h post-glucose load.
[a] Valus are means ± SDs for normally distributed data, median (IQRs) for nonnormally distributed data, or n (%) for categorial data. Comparison between case-controls were performed with Student's t test or Mann–Whitney U test for continuous variables, and chi-square test for categorical variables.

**Table 2**
Association between plasma Cu concentrations and GDM.[a]

| | Quartiles of plasma Cu concentration | | | | *P* value for trend[d] | Per SD increment of plasma Cu |
|---|---|---|---|---|---|---|
| | Quartile 1 (≤1594.27 μg/L) | Quartile 2 (1594.28 –1809.89 μg/L) | Quartile 3 (1809.90 –2064.04 μg/L) | Quartile 4 (>2064.04 μg/L) | | |
| No. of GDM cases/ controls | 34/62 | 50/62 | 74/62 | 90/62 | | |
| Crude model | 1 | 1.60 (0.89–2.85) | 2.44 (1.35–4.39) | 3.01 (1.68–5.38) | <0.001 | 1.40 (1.15–1.71) |
| Model 1[b] | 1 | 1.70 (0.88–3.29) | 2.44 (1.24–4.78) | 2.72 (1.40–5.27) | <0.001 | 1.31 (1.05–1.64) |
| Model 2[c] | 1 | 1.79 (0.90–3.55) | 2.72 (1.35–5.48) | 2.91 (1.48–5.75) | <0.001 | 1.33 (1.06–1.67) |

Abbreviations: Cu, copper; GDM, gestational diabetes mellitus; SD, standard deviation.
[a] Values are ORs (95% CIs).
[b] Model 1 adjusted for age (years) and prepregnancy body mass index (kg/m$^2$).
[c] Model 2 adjusted for model 1 plus gestational age at blood drawing (weeks), parity, family history of diabetes (yes/no), drinking habits (yes/no) and smoking (yes/no).
[d] Tests for linear trend were conducted by using the median value for each quartile and treating it as a continuous variable in the conditional logistic regression.

**Table 3**
Correlation coefficients between Cu and other variables of interest.

| Variables | Unadjusted | | Adjusted[a] | |
|---|---|---|---|---|
| | *r* | *P* | *r* | *P* |
| Fasting plasma glucose, mmol/L | 0.203 | <0.001 | 0.147 | 0.001 |
| OGTT-1h, mmol/L | 0.113 | 0.012 | 0.092 | 0.043 |
| OGTT-2h, mmol/L | 0.126 | 0.005 | 0.141 | 0.002 |
| Fasting plasma insulin, μU/mL | 0.157 | <0.001 | 0.053 | 0.245 |
| HOMA-IR | 0.187 | <0.001 | 0.068 | 0.135 |
| Total cholesterol, mmol/L | 0.053 | 0.237 | 0.072 | 0.114 |
| Triglycerides, mmol/L | 0.096 | 0.033 | 0.067 | 0.141 |
| LDL cholesterol, mmol/L | 0.045 | 0.317 | 0.050 | 0.274 |
| HDL cholesterol, mmol/L | −0.037 | 0.414 | 0.017 | 0.703 |

Abbreviations: Cu, copper; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low density lipoprotein; OGTT-1h, 1-h post-glucose load; OGTT-2h, 2-h post-glucose load.
[a] Partial correlations, adjusted for age (years), prepregnancy body mass index (kg/m$^2$), gestational age at blood drawing (weeks), parity, family history of diabetes (yes/no), drinking habits (yes/no) and smoking (yes/no).

previous studies [16,19,31,32], which made our results more comparable to other studies.

There is biologic plausibility for an important role of Cu in GDM risk. First, ROS is thought to contribute to the development of insulin resistance [35]. Cu ion can facilitate ROS production by mediating the transfer of electrons, and it has high reactivity in the oxidation reduction reactions [36]. High levels of Cu ion and ROS have been found in diabetic C57BL/KsJ-db/db mice, then treatment with Cu chelating agent reduced ROS levels, and consequently ameliorated glucose and lipid metabolism [13]. These findings indicated that Cu might be involved in the pathogenesis of glucose metabolic disorders through ROS generation. Second, Cu can increase the rate of advanced glycation end-products (AGEs) formation, which is associated with diabetes and its complications [37].

There is evidence that treatment of diabetic rats with Cu chelator could significantly reduce the levels of serum Cu as well as AGEs and AGE-precursors [38]. AGEs are deemed to produce the pathological $Cu^{2+}$-binding sites in tissues, once AGEs-mediated $Cu^{2+}$ binding is initiated, a feedforward process of $Cu^{2+}$ catalysis becomes activated, thus further enhance the AGEs-modification and $Cu^{2+}$ trapping potential [39]. Third, due to its ability to directly bind proteins and nucleic acids, Cu plays a role in oxidative modification reactions *in situ* and the occurrence of protein crosslinking, which could lead to impaired activity [40]. Forth, Cu ion was found to have the ability to stimulate the aggregation of human amylin peptide into amyloid fibril [41], which is associated with reduced β-cell mass and progressive islet β-cell failure [42]. In the meanwhile, the by-product $H_2O_2$ generated during the process could also induce damaging oxidation, cell toxicity and progressive β-cells degeneration [43].

Although our study found a positive relationship between plasma Cu concentrations and GDM odds, the role of Cu in any causal or compensatory pathway is still unclear. Diabetic status may also have some influence on Cu metabolism and ultimately affect Cu uptake or distribution [44]. As key proteins for Cu transport, Cu(I)-ATPases (ATP7A and ATP7B proteins) were found to be involved in diabetes pathogenesis. Animal study conducted by Zhang et al. demonstrated that diabetes depressed levels of ATP7B protein and also altered its localization in left ventricular samples, whereas Cu selective chelator elevated ATP7A [45]. Another group found that physiological concentrations of insulin increased ATP7B activity in cultured hepatic cells and in tissues while glucagon had opposite effect [46]. In addition, according to previous study, patients with hyperglycemia were showed to have increased glycated and less active Cu, Zn-SOD [47]. A site-specific and random fragmentation of human Cu, Zn-SOD was observed following the glycation reaction, leading to the inactivation of the enzyme and a time-dependent release of $Cu^{2+}$, which exacerbated oxidative stress and insulin resistance in turn [48]. More studies are needed to help understand the possible mechanisms involved in the disruption of Cu homeostasis in patients with glucose metabolic disorders.

Our study displayed various strengths. As far as we know, this is the first study systematically investigating the association between plasma Cu concentrations and odds of GDM in a relatively large sample size. The diagnosis of GDM was made by an OGTT and all the participants were free of former diagnosis, because treatments like lifestyle changes may alter the status of Cu metabolism and distort the association. Moreover, each case was well matched to one control by age, gestational age at blood drawing and parity to minimize the influence of these major confounders.

Several limitations of our study should also be acknowledged. First, the case–control nature of our study disenabled us to infer any causality relationship. Second, the participants in this study were from a specific city in China, which limits the generalizability of our findings to other populations. However, the relative homogeneity of this study population in ethnic background and copper environmental exposure enhances the internal validity of our findings. Third, dietary intake data was not available in our study, which disenabled us to conduct the analysis between dietary factors and plasma Cu. However, circulating Cu is an accepted biomarker of Cu intake and is able to objectively assess dietary consumption without the bias of self-reported dietary intake errors [49,50]. Furthermore, even that we have controlled for various confounding factors like age, prepregnancy BMI, gestational age and parity, there might be other residual confounding that we did not measure but may impact the association examined, such as supplements use during pregnancy or morning sickness.

In conclusion, our study indicated a significantly increased odds of GDM in association with higher concentrations of plasma Cu. Prospective cohort studies in other populations are needed to confirm our findings and explorations of the role of Cu in GDM pathogenesis should be undertaken using in vivo and in vitro models.

## Conflicts of interest

The authors declare that they have no competing interests.

## Statement of authorship

PL and LL designed the study; PL, JY, YZ, SL, SC, JW and QS contributed to the data collection; PL and TS interpreted and analyzed the data; PL and WY wrote the manuscript; ZS and XL edited the manuscript; all authors read and approved the final manuscript. LL and WY are the guarantors of this work and have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

## Funding sources

This work was funded by the Major International (Regional) Joint Research Project (NSFC8181001139), the National Key Research and Development Program of China (2017YFC1600500), the National Natural Science Foundation of China (81703214 and 21537001). The funding sources had no role in study design; in collection, analysis and interpretation of the data; in writing and reviewing of the manuscript; and in decision to submit the article for publication.

## Acknowledgements

The authors gratefully thank Tingting Zhu (Zhuhai Center for Disease Control and Prevention, Zhuhai, China) and Hongfei Ma (Wuhan Center for Disease Control and Prevention, Wuhan, China) for their hard work on blood sample collection. The authors also acknowledged the cooperation of the pregnant women who took part in this study.

## References

[1] International Diabetes Federation. IDF diabetes atlas. 8th ed. 2018. http://www.diabetesatlas.org/. [Accessed 5 November 2018].
[2] Farrar D, Simmonds M, Bryant M, Sheldon TA, Tuffnell D, Golder S, et al. Hyperglycaemia and risk of adverse perinatal outcomes: systematic review and meta-analysis. BMJ 2016;354:i4694. https://doi.org/10.1136/bmj.i4694.
[3] Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet 2009;373:1773–9. https://doi.org/10.1016/s0140-6736(09)60731-5.
[4] Fetita LS, Sobngwi E, Serradas P, Calvo F, Gautier JF. Consequences of fetal exposure to maternal diabetes in offspring. J Clin Endocrinol Metabol 2006;91:3718–24. https://doi.org/10.1210/jc.2006-0624.
[5] Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: risks and management during and after pregnancy. Nat Rev Endocrinol 2012;8:639–49. https://doi.org/10.1038/nrendo.2012.96.
[6] Arredondo M, Nunez MT. Iron and copper metabolism. Mol Aspect Med 2005;26:313–27. https://doi.org/10.1016/j.mam.2005.07.010.
[7] Squitti R, Siotto M, Polimanti R. Low-copper diet as a preventive strategy for Alzheimer's disease. Neurobiol Aging 2014;35(Suppl. 2):S40–50. https://doi.org/10.1016/j.neurobiolaging.2014.02.031.
[8] Lutsenko S. Human copper homeostasis: a network of interconnected pathways. Curr Opin Chem Biol 2010;14:211–7. https://doi.org/10.1016/j.cbpa.2010.01.003.
[9] Gambling L, Kennedy C, McArdle HJ. Iron and copper in fetal development. Semin Cell Dev Biol 2011;22:637–44. https://doi.org/10.1016/j.semcdb.2011.08.011.
[10] Qiu Q, Zhang F, Zhu W, Wu J, Liang M. Copper in diabetes mellitus: a meta-analysis and systematic review of plasma and serum studies. Biol Trace Elem Res 2017;177:53–63. https://doi.org/10.1007/s12011-016-0877-y.

[11] Lin CC, Huang HH, Hu CW, Chen BH, Chong IW, Chao YY, et al. Trace elements, oxidative stress and glycemic control in young people with type 1 diabetes mellitus. J Trace Elem Med Biol 2014;28:18–22. https://doi.org/10.1016/j.jtemb.2013.11.001.
[12] Naka T, Kaneto H, Katakami N, Matsuoka TA, Harada A, Yamasaki Y, et al. Association of serum copper levels and glycemic control in patients with type 2 diabetes. Endocr J 2013;60:393–6.
[13] Tanaka A, Kaneto H, Miyatsuka T, Yamamoto K, Yoshiuchi K, Yamasaki Y, et al. Role of copper ion in the pathogenesis of type 2 diabetes. Endocr J 2009;56:699–706.
[14] Trigwell SM, Radford PM, Page SR, Loweth AC, James RF, Morgan NG, et al. Islet glutamic acid decarboxylase modified by reactive oxygen species is recognized by antibodies from patients with type 1 diabetes mellitus. Clin Exp Immunol 2001;126:242–9.
[15] Ma L, Li X, Wang Y, Zheng W, Chen T. Cu(II) inhibits hIAPP fibrillation and promotes hIAPP-induced beta cell apoptosis through induction of ROS-mediated mitochondrial dysfunction. J Inorg Biochem 2014;140:143–52. https://doi.org/10.1016/j.jinorgbio.2014.07.002.
[16] Choi R, Sun J, Yoo H, Kim S, Cho YY, Kim HJ, et al. A prospective study of serum trace elements in healthy Korean pregnant women. Nutrients 2016;8. https://doi.org/10.3390/nu8110749.
[17] Ryan EA. Hormones and insulin resistance during pregnancy. Lancet 2003;362:1777–8. https://doi.org/10.1016/s0140-6736(03)14942-2.
[18] Uriu-Adams JY, Scherr RE, Lanoue L, Keen CL. Influence of copper on early development: prenatal and postnatal considerations. Biofactors 2010;36:136–52. https://doi.org/10.1002/biof.85.
[19] Wang Y, Tan M, Huang Z, Sheng L, Ge Y, Zhang H, et al. Elemental contents in serum of pregnant women with gestational diabetes mellitus. Biol Trace Elem Res 2002;88:113–8. https://doi.org/10.1385/bter:88:2:113.
[20] Loven A, Romem Y, Pelly IZ, Holcberg G, Agam G. Copper metabolism–a factor in gestational diabetes? Clin Chim Acta 1992;213:51–9.
[21] Al-Saleh E, Nandakumaran M, Al-Rashdan I, Al-Harmi J, Al-Shammari M. Maternal-foetal status of copper, iron, molybdenum, selenium and zinc in obese gestational diabetic pregnancies. Acta Diabetol 2007;44:106–13. https://doi.org/10.1007/s00592-007-0250-x.
[22] Akhlaghi F, Bagheri SM, Rajabi O. A comparative study of relationship between micronutrients and gestational diabetes. ISRN Obstet Gynecol 2012;2012:470419. https://doi.org/10.5402/2012/470419.
[23] American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. Diabetes Care 2018;41:S13–27. https://doi.org/10.2337/dc18-S002.
[24] Li P, Zhong C, Li S, Sun T, Huang H, Chen X, et al. Plasma concentration of trimethylamine-N-oxide and risk of gestational diabetes mellitus. Am J Clin Nutr 2018;108:603–10. https://doi.org/10.1093/ajcn/nqy116.
[25] Sridhar SB, Xu F, Darbinian J, Quesenberry CP, Ferrara A, Hedderson MM. Pregravid liver enzyme levels and risk of gestational diabetes mellitus during a subsequent pregnancy. Diabetes Care 2014;37:1878–84. https://doi.org/10.2337/dc13-2229.
[26] Solomon CG, Willett WC, Carey VJ, Rich-Edwards J, Hunter DJ, Colditz GA, et al. A prospective study of pregravid determinants of gestational diabetes mellitus. JAMA 1997;278:1078–83.
[27] Healy J, Tipton K. Ceruloplasmin and what it might do. J Neural Transm (Vienna) 2007;114:777–81. https://doi.org/10.1007/s00702-007-0687-7.
[28] Eshak ES, Iso H, Maruyama K, Muraki I, Tamakoshi A. Associations between dietary intakes of iron, copper and zinc with risk of type 2 diabetes mellitus: a large population-based prospective cohort study. Clin Nutr 2018;37:667–74. https://doi.org/10.1016/j.clnu.2017.02.010.
[29] Stepien M, Jenab M, Freisling H, Becker NP, Czuban M, Tjonneland A, et al. Pre-diagnostic copper and zinc biomarkers and colorectal cancer risk in the European prospective investigation into cancer and nutrition cohort. Carcinogenesis 2017;38:699–707. https://doi.org/10.1093/carcin/bgx051.
[30] El Ati-Hellal M, Doggui R, Hedhili A, Traissac P, El Ati J. Zinc and copper status in childbearing age Tunisian women: relation to age, residential area, socio-economic situation and physiologic characteristics. Chemosphere 2016;149:231–7. https://doi.org/10.1016/j.chemosphere.2016.01.100.
[31] Kilinc M, Coskun A, Bilge F, Imrek SS, Atli Y. 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 2010;24:152–6. https://doi.org/10.1016/j.jtemb.2010.01.004.
[32] Reyes H, Baez ME, Gonzalez MC, Hernandez I, Palma J, Ribalta J, et al. Selenium, zinc and copper plasma levels in intrahepatic cholestasis of pregnancy, in normal pregnancies and in healthy individuals, in Chile. J Hepatol 2000;32:542–9.
[33] Stephens TV, Woo H, Innis SM, Elango R. Healthy pregnant women in Canada are consuming more dietary protein at 16- and 36-week gestation than currently recommended by the dietary reference intakes, primarily from dairy food sources. Nutr Res 2014;34:569–76. https://doi.org/10.1016/j.nutres.2014.07.001.
[34] Arredondo M, Nunez H, Lopez G, Pizarro F, Ayala M, Araya M. Influence of estrogens on copper indicators: in vivo and in vitro studies. Biol Trace Elem Res 2010;134:252–64. https://doi.org/10.1007/s12011-009-8475-x.
[35] Loh K, Deng H, Fukushima A, Cai X, Boivin B, Galic S, et al. Reactive oxygen species enhance insulin sensitivity. Cell Metabol 2009;10:260–72. https://doi.org/10.1016/j.cmet.2009.08.009.
[36] Malmstrom BG, Leckner J. The chemical biology of copper. Curr Opin Chem Biol 1998;2:286–92.
[37] Uribarri J, Cai W, Ramdas M, Goodman S, Pyzik R, Chen X, et al. Restriction of advanced glycation end products improves insulin resistance in human type 2 diabetes: potential role of AGER1 and SIRT1. Diabetes Care 2011;34:1610–6. https://doi.org/10.2337/dc11-0091.
[38] Hamada Y, Nakashima E, Naruse K, Nakae M, Naiki M, Fujisawa H, et al. A copper chelating agent suppresses carbonyl stress in diabetic rat lenses. J Diabetes Complicat 2005;19:328–34. https://doi.org/10.1016/j.jdiacomp.2005.08.002.
[39] Kamalov M, Harris PW, Hartinger CG, Miskelly GM, Cooper GJ, Brimble MA. Physicochemical studies on the copper(II) binding by glycated collagen telopeptides. Org Biomol Chem 2015;13:3058–63. https://doi.org/10.1039/c4ob02536a.
[40] Cecconi I, Scaloni A, Rastelli G, Moroni M, Vilardo PG, Costantino L, et al. Oxidative modification of aldose reductase induced by copper ion. Definition of the metal-protein interaction mechanism. J Biol Chem 2002;277:42017–27. https://doi.org/10.1074/jbc.M206945200.
[41] Masad A, Hayes L, Tabner BJ, Turnbull S, Cooper LJ, Fullwood NJ, et al. Copper-mediated formation of hydrogen peroxide from the amylin peptide: a novel mechanism for degeneration of islet cells in type-2 diabetes mellitus? FEBS Lett 2007;581:3489–93. https://doi.org/10.1016/j.febslet.2007.06.061.
[42] Ritzel RA, Meier JJ, Lin CY, Veldhuis JD, Butler PC. Human islet amyloid polypeptide oligomers disrupt cell coupling, induce apoptosis, and impair insulin secretion in isolated human islets. Diabetes 2007;56:65–71. https://doi.org/10.2337/db06-0734.
[43] Hoppener JW, Lips CJ. Role of islet amyloid in type 2 diabetes mellitus. Int J Biochem Cell Biol 2006;38:726–36. https://doi.org/10.1016/j.biocel.2005.12.009.
[44] Lowe J, Taveira-da-Silva R, Hilario-Souza E. Dissecting copper homeostasis in diabetes mellitus. IUBMB Life 2017;69:255–62. https://doi.org/10.1002/iub.1614.
[45] Zhang S, Liu H, Amarsingh GV, Cheung CC, Hogl S, Narayanan U, et al. Diabetic cardiomyopathy is associated with defective myocellular copper regulation and both defects are rectified by divalent copper chelation. Cardiovasc Diabetol 2014;13:100. https://doi.org/10.1186/1475-2840-13-100.
[46] Hilario-Souza E, Cuillel M, Mintz E, Charbonnier P, Vieyra A, Cassio D, et al. Modulation of hepatic copper-ATPase activity by insulin and glucagon involves protein kinase A (PKA) signaling pathway. Biochim Biophys Acta 2016;1862:2086–97. https://doi.org/10.1016/j.bbadis.2016.08.008.
[47] Kawamura N, Ookawara T, Suzuki K, Konishi K, Mino M, Taniguchi N. Increased glycated Cu,Zn-superoxide dismutase levels in erythrocytes of patients with insulin-dependent diabetis mellitus. J Clin Endocrinol Metabol 1992;74:1352–4. https://doi.org/10.1210/jcem.74.6.1592880.
[48] Ookawara T, Kawamura N, Kitagawa Y, Taniguchi N. Site-specific and random fragmentation of Cu,Zn-superoxide dismutase by glycation reaction. Implication of reactive oxygen species. J Biol Chem 1992;267:18505–10.
[49] Bo S, Durazzo M, Gambino R, Berutti C, Milanesio N, Caropreso A, et al. Associations of dietary and serum copper with inflammation, oxidative stress, and metabolic variables in adults. J Nutr 2008;138:305–10. https://doi.org/10.1093/jn/138.2.305.
[50] Harvey LJ, Ashton K, Hooper L, Casgrain A, Fairweather-Tait SJ. Methods of assessment of copper status in humans: a systematic review. Am J Clin Nutr 2009;89. https://doi.org/10.3945/ajcn.2009.27230E. 2009s-24s.