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Original Investigation

The first trimester plasma copper–zinc ratio is independently related to pregnancy-specific psychological distress symptoms throughout pregnancy



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ABSTRACT

Objectives: High plasma copper (Cu) and low zinc (Zn) levels have been associated with depression. However, most studies used low sample sizes and a cross-sectional design, and perinatal data are scarce. We investigated the possible association between pregnancy-specific psychological distress and the plasma CuZn ratio using a prospective design.

Methods: Pregnancy-specific distress symptoms were assessed at each trimester by means of the Tilburg Pregnancy Distress Scale, negative affect subscale, in 2036 pregnant women. Cu and Zn were assessed at 12 wk of gestation in plasma samples by inductively coupled plasma mass spectrometry. Growth mixture modeling determined trajectories of women's pregnancy-specific negative affect (P-NA) symptoms, which were entered in a multiple logistic regression analysis as dependent variable and the CuZn ratio as independent variable.

Results: Two P-NA symptom classes were found: 1) persistently low ($n = 1820$) and 2) persistently high ($n = 216$). A higher CuZn ratio was independently associated with persistently high P-NA symptom scores (odds ratio = 1.52; 95% confidence interval, 1.13–2.04) after adjustment for confounders. A sensitivity analysis was performed excluding all women with high P-NA scores at 12 wk of gestation (>1 SD above the mean P-NA score). In the 1719 remaining women, a higher CuZn ratio significantly predicted the development of increasing P-NA symptom scores after adjustment for confounders (odds ratio = 1.40; 95% confidence interval, 1.04–1.95).

Conclusions: A higher CuZn plasma ratio is an independent determinant of developing pregnancy-specific distress symptoms throughout pregnancy, suggesting that micronutrients could be used as novel biomarkers for psychological distress research of perinatal mood disorders.

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Introduction

Psychological distress occurs in about 25% of pregnant women [1,2] and can be defined as “a state of emotional suffering characterized by symptoms of depression and anxiety sometimes accompanied by somatic symptoms” [3]. Psychological distress has been

associated with impaired obstetric outcome, including intrauterine growth retardation, preterm birth, and low birth weight [4–6]. Pregnancy-specific distress on the other hand, reflects pregnancy-specific worries that have been described as “a woman's response to the transition to motherhood, which includes changes to their bodies, roles, relationships and social circumstances, birth experiences, and the demands, challenges, losses and gains associated with being a new mother” [7]. It presents the main focus of the present study. Some metals, such as zinc (Zn) and copper (Cu), are

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essential nutrients with roles in the context of infection, immune function, and inflammatory responses. They also play an important role in neurochemical mechanisms and in several psychiatric conditions, such as depression, anxiety, autism, and schizophrenia, which have been associated with altered concentrations of trace elements [8]. For example, Zn homeostasis is crucial for adequate functioning of brain cells of the hippocampus, amygdala, and cortex, all involved in the pathophysiology of depression [9–12]. Cu also is involved in psycho-immunologic mechanisms resulting in mental disorders [13]. In a recent case-control study including 247 patients with major depressive disorder, the plasma Zn concentration was significantly decreased, whereas the Cu concentration was significantly increased compared with healthy volunteers [14]. Thus, the CuZn ratio combines alterations of both micronutrients and may constitute a meaningful diagnostic biomarker for psychological distress and changes observed in diseases and ageing [15]. Few studies on a possible association with pregnancy-specific distress exist. Pregnancy-specific distress is multifactorial in terms of etiology and heterogeneous regarding its behavioral and biological correlates, with a large variability in symptom profiles both in and between individuals over time [16]. A statistical method that addresses with these individual differences in symptoms over time is growth mixture modeling. We used this method to classify individuals into trajectory classes, based on similarities in the course of symptom profiles rather than differences [17,18]. The first aim of the present study was to evaluate the relation between Zn and Cu plasma levels at 12 wk of gestation in relation to the acute-phase protein C-reactive protein (CRP). The second aim of the study was to evaluate the association between different Zn and Cu plasma concentrations and different trajectories of pregnancy-specific distress symptoms throughout pregnancy.

Material and methods

Participants and procedures

The present prospective study sample is a subsample of women participating in the Holistic Approach to Pregnancy and the first Postpartum Year study, the details of which have been described previously [19]. The following exclusion criteria were used: a multiple pregnancy, taking antidepressant medication before 20 wk of pregnancy, previous history of severe psychiatric disorders (e.g., schizophrenia, borderline personality disorder, or bipolar disorder), and a documented history of chronic disease (e.g., type 1 diabetes mellitus or thyroid dysfunction). Eligibility criteria included enrollment during the first 14 wk of pregnancy and sufficient understanding of the Dutch language to complete the questionnaires. Of the 3160 eligible women who were approached, 2219 (74%) participated and provided written informed consent. Of these women, all psychological, obstetric, and biological data needed for the present study were available in 2036 women. The study was approved by the local medical ethics and the psychology ethics committees (protocol no. EC-2012.25).

Measures

At 12 wk of gestation, non-fasting blood samples were taken, and women completed a set of questionnaires.

Pregnancy-specific distress

Pregnancy-specific distress was measured with the Tilburg Pregnancy Distress Scale at 12, 22 and 32 wk of gestation [20]. The scale consists of two subscales: negative affect (NA), 11 items, and partner involvement, 5 items, of which only the NA subscale was used in the present study. The NA subscale assesses worries related to pregnancy, childbirth, and the postpartum period, and scores range from 0 to 33, with higher scores reflecting more pregnancy-specific NA. Therefore, we use the term pregnancy-specific NA (P-NA) throughout this article. The Tilburg Pregnancy Distress Scale has been found to be a reliable and valid instrument, with a Cronbach α of 0.78 for the total scale and a Cronbach α of 0.80 for the NA subscale [20]. The scale has been evaluated as excellent regarding the internal consistency [21].

Characteristics

The demographic characteristics, obstetric features, lifestyle habits, and psychiatric history of participating women were assessed at 12 wk of gestation (Table 1).

Zinc and copper

Plasma Zn and Cu concentrations were measured at 12 wk of gestation in heparinized plasma samples by inductively coupled plasma mass spectrometry using a NexION 300X instrument (PerkinElmer, Groningen, the Netherlands) in the kinetic energy discrimination mode. Zn and Cu were measured by employing helium at a flow rate of 1.0 mL/min as the kinetic energy discrimination gas to remove polyatomic interferences. Within- and between-run variations were assessed by Clinical and Laboratory Standards Institute guideline EP5-A2 and found <3% for low and high plasma concentrations of Zn and Cu. According to Clinical and Laboratory Standards Institute guideline EP 17-A2, the lower limit of quantitation was calculated at 0.30 and 0.27 $\mu\text{mol/L}$ for Zn and Cu, respectively. Certified reference materials Seronorm L-1 or L-2 (Nycomed, Norway, Oslo) were used to monitor accuracy. During measurement of the samples of this study, for L-1 (lot 1309438), values for Zn and Cu were 17.4 $\mu\text{mol/L}$ (reported analytical value 16.8 $\mu\text{mol/L}$ and range 14.6–19.0 $\mu\text{mol/L}$) and 17.9 $\mu\text{mol/L}$ (reported analytical value 17.1 $\mu\text{mol/L}$ and range 15.7–18.5 $\mu\text{mol/L}$), respectively. For L-2 (lot 1309416), values for Zn and Cu were 25.7 $\mu\text{mol/L}$ (reported certified mean 24.7 $\mu\text{mol/L}$ and range 21.5–28.0 $\mu\text{mol/L}$) and 29.4 $\mu\text{mol/L}$ (reported certified mean 29.1 $\mu\text{mol/L}$ and range 26.7–31.5 $\mu\text{mol/L}$), respectively. Moreover, pooled plasma samples were measured as well to monitor within-run variation at low and high concentrations. A cutoff <5th percentile was used to define a low Zn concentration, and a cutoff >95th percentile was used to define an elevated Cu concentration.

C-reactive protein

CRP levels were assessed at 12 wk gestation in lithium-heparin plasma samples using a particle-enhanced immunoturbidimetric assay (CRP 3, cobas c501 platform; Roche Diagnostics, Mannheim, Germany). The measurement range of the assay is 0.3 to 350 mg/L. Within-laboratory coefficients of variation were 2.1% and 1.7% at levels of 7.3 and 50 mg/L, respectively. Values >10 mg/L were rounded to the nearest whole number. A cutoff >95th percentile was used to define an elevated CRP concentration.

Statistics

The most recent guidelines of the Clinical and Laboratory Standards Institute for defining adequate cutoffs in the clinical laboratory advocate, when applying single cutoffs, that the 5th or the 95th percentile can be used to define the lower or the higher reference limit, respectively [22].

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Zn and Cu levels were normally distributed but because CRP was not normally distributed, we used log-transformed CRP to determine Spearman correlations with Zn, Cu, and CuZn. Mann-Whitney *U* tests were used to compare CRP between subgroups of women (with scores above and below cutoff for low Zn, high Cu, and high CuZn). In addition, χ^2 tests were used to compare the number of women with a high CRP (>95th percentile) between these subgroups of women.

Growth mixture modeling was conducted using Mplus version 8.7 [23] to determine trajectories of women's P-NA symptoms across three time points (12, 22, and 32 wk of gestation), using the P-NA total scores. Because three time points were included, we could estimate only linear growth factors. After a one-class model, models with increasing numbers of classes were fitted. Lower Bayesian information criterion values, and a significant Lo-Mendell-Rubin likelihood ratio test and bootstrap likelihood ratio test were considered fit indices to obtain the optimal number of classes [17,24,25]. Moreover, the entropy was considered, in which a value closer to 1 indicates a more optimal fit of participants in their respective class and a clearer delineation of classes [24]. In addition, each class was required to include >1% of the total sample [17]. The class membership of all participants corresponding to the model that had the best model fit for P-NA symptoms throughout pregnancy was exported to SPSS for subsequent analyses.

Finally, a multiple logistic regression analysis was performed with P-NA trajectories as the dependent variables and the CuZn ratio as independent variable. Data are presented as odds ratios (ORs) with 95% confidence intervals (CIs). We adjusted for four possible covariates based on previous literature, namely parity, unplanned pregnancy, prepregnancy body mass index, and previous diagnosis of depression.

Results

Zinc, copper, copper-zinc ratio, and C-reactive protein

Table 1 lists the characteristics of the study sample ($n = 2036$) as well as the values of Zn, Cu, CuZn ratio, and CRP. A majority of the

Table 1
Characteristics of 2036 women with assessment of Zn, Cu, CuZn ratio, and CRP at 12 wk of gestation

| Characteristics | n (%) | Mean (SD) | Range | Median (IQR) |
|--|-------------|------------|------------|------------------|
| Demographic features | | | | |
| Dutch | 1928 (97.7) | | | |
| Age (y) | | 30.4 (3.7) | 19–43 | |
| High level of education | 1241 (63.2) | | | |
| Partner | 2006 (98.5) | | | |
| Paid job | 1886 (92.6) | | | |
| Obstetric features | | | | |
| Primiparous | 1011 (49.7) | | | |
| Previous miscarriage | 525 (25.8) | | | |
| Current unplanned pregnancy | 128 (6.3) | | | |
| Lifestyle habits during pregnancy | | | | |
| Smoking | 133 (6.5) | | | |
| Any alcohol intake | 77 (3.8) | | | |
| Pre-pregnancy BMI | | 24.0 (4.0) | 16.0–42.5 | |
| Psychiatric history | | | | |
| Previous diagnosis of depression | 328 (16.1) | | | |
| Trace elements | | | | |
| Zn | | 12.6 (1.8) | 7.5–20.9 | 12.4 (11.3–13.7) |
| Low Zn: < 5th percentile: < 9.97 $\mu\text{mol/L}$ | 102 (5.0) | | | |
| Cu | | 26.3 (4.7) | 12.8–47.8 | 25.9 (23.0–29.2) |
| High Cu: > 95th percentile: > 34.3 $\mu\text{mol/L}$ | 101 (5.0) | | | |
| CuZn ratio | | 2.1 (0.5) | 0.98–4.0 | 2.1 (1.8–2.4) |
| High CuZn ratio: > 95th percentile: > 3.05 | 102 (5.0) | | | |
| CRP | | 5.4 (6.4) | 0.06–106.0 | 3.7 (2.0–6.4) |
| High CRP: > 95th percentile: > 16.0 mg/L | 111 (5.5) | | | |

BMI, body mass index; Cu, Copper; CuZn, copper-zinc ratio; CRP, C-reactive protein; High level of education, bachelor's degree or higher; IQR, interquartile range; Zn, zinc

women were Dutch (97.7%), had a partner (98.5%), and had a paid job (92.6%). Primiparous and multiparous women were almost equally distributed (49.7% versus 50.3%). Of the 102 women with a high CuZn ratio (>95th percentile), 41 (40%) women had a low Zn concentration (<5th percentile) and 38 (37%) a high Cu concentration (>95th percentile). LogCRP was significantly correlated with Cu ($r = 0.50$; $P < 0.001$) as well as with the CuZn ratio ($r = 0.42$; $P < 0.001$), whereas it was inversely correlated with Zn ($r = -0.05$; $P = 0.023$). In Table 2, we compared the CRP levels between women below and above the cutoffs used to define low Zn, high Cu, and high CuZn ratio. Moreover, we compared the number of women

with CRP levels >95th percentile cutoff in these subgroups. The women with low Zn values (<5th percentile) had significantly higher CRP levels compared with those above this cutoff (Mann-Whitney U test: z score [1] = 3.5; $P < 0.001$). The number of women with an elevated CRP in the low Zn group was 8.8% compared with 5.3% in the normal Zn subgroup (χ^2 [1] = 2.4; $P = 0.124$). The women of the high Cu group (>95th percentile) had a significantly higher CRP level compared with the women with Cu below this cutoff (Mann-Whitney U test: z score [1] = 11.2; $P < 0.001$). In addition, the women of this high Cu subgroup included significantly more women with elevated CRP (31.7%) compared with the normal Cu level subgroup (4.1%, χ^2 [1] = 141.9; $P < 0.001$). The women with a high CuZn ratio had significantly higher CRP levels compared with those below the cutoff (Mann-Whitney U test: z score [1] = 9.5; $P < 0.001$) and included significantly more women with high CRP compared with the normal CuZn ratio group: 23.5% versus 4.5% (χ^2 [1] = 68.1; $P < 0.001$). At a univariate level, the CuZn ratio was positively correlated with body mass index ($r = 0.25$; $P < 0.001$), however, with small effect size.

We subsequently compared the mean P-NA symptom scores at three trimesters between subgroups according to the low Zn cutoff (<5th percentile), high Cu cutoff (>95th percentile), and high CuZn cutoff (>95th percentile) using generalized linear model–analysis of variance. Low Zn was not significantly associated with the mean P-NA symptom scores at different trimesters (F test [1] = 1.1; $P = 0.29$), whereas high Cu was significantly related to differences in mean P-NA symptom scores (F test [1] = 6.6; $P = 0.010$) with the highest difference for the high CuZn ratio cutoff: F test [1] = 14.1; $P < 0.001$). Therefore, we continued further analyses focusing on the CuZn ratio. Of the 102 women with a CuZn ratio >95th percentile, 42 (41.2%) had a Zn concentration <5th percentile, whereas 38 (37.3%) had a Cu level >95th percentile (Table 1). We found no association between high CRP levels at 12 wk of gestation (>95th percentile) and mean P-NA symptom scores at different trimesters (generalized linear model–analysis of variance: F [1] = 0.003; $P = 0.960$).

Table 2
CRP distribution according to subgroups of women with low Zn, high Cu, and high CuZn ratio versus subgroups with normal Zn, Cu, and CuZn ratio, respectively ($n = 2036$)

| Trace elements | CRP | High CRP: > 95th percentile: > 16.0 mg/L |
|--|-----------------------------|--|
| | Median (IQR) | n (%) |
| Zn | | |
| Low: <5th percentile: <9.97 $\mu\text{mol/L}$ | 4.7 (3.2–7.8) ^a | 9 (8.8) |
| Normal: ≥ 9.97 $\mu\text{mol/L}$ | 3.7 (2.0–6.3) ^a | 102 (5.3) |
| Cu | | |
| High: >95th percentile: >34.3 $\mu\text{mol/L}$ | 9.9 (6.2–17.5) [*] | 32 (31.7) [*] |
| Normal: ≤ 34.3 $\mu\text{mol/L}$ | 3.6 (2.0–6.1) [*] | 79 (4.1) [*] |
| CuZn | | |
| High: >95th percentile: >3.05 | 8.5 (4.8–15.0) [*] | 24 (23.5) [*] |
| Normal: ≤ 3.05 | 3.6 (2.0–6.1) [*] | 87 (4.5) [*] |

CRP, C-reactive protein; Cu, copper; CuZn, copper-zinc ratio; IQR, interquartile range; Zn, zinc

Mann-Whitney U test to compare CRP between subgroups and χ^2 test to compare number of women with high CRP between subgroups

^{*} $P < 0.001$.

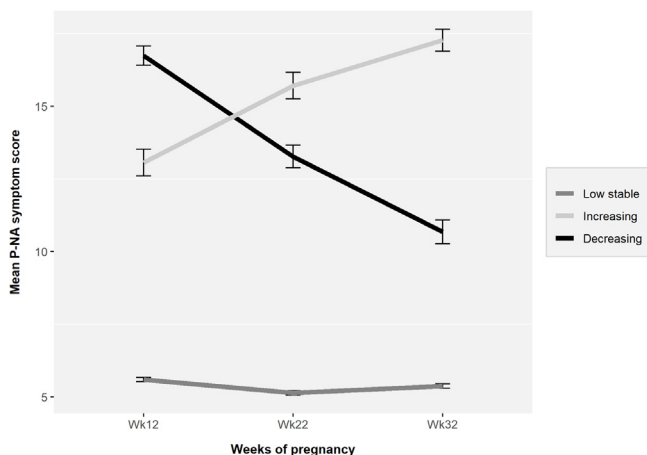


Fig. 1. Longitudinal trajectories of pregnancy-specific negative affect (P-NA) symptoms during pregnancy ($n = 2036$). Mean (SD) P-NA symptom scores per trimester were: Low stable class, 5.6 (3.1), 5.1 (3.1), and 5.4 (3.2); Increasing class, 13.1 (4.7), 15.7 (4.7), and 17.3 (3.8); and Decreasing class, 16.7 (3.5), 13.3 (4.0), and 10.7 (4.0).

Trajectories of pregnancy-specific negative affect symptoms and copper-zinc ratio

Growth mixture modeling found that a three-class solution fits the data appropriately, based on the fit indices (bayesian information criterion, Lo-Mendell-Rubin likelihood ratio test, and bootstrap likelihood ratio test) and entropy (Supplementary Table 1), resulting in three different trajectories of P-NA symptoms throughout pregnancy (Fig. 1). The reference group consisted of 1820 (89.4%) women reporting low stable P-NA symptom scores throughout pregnancy. The increasing trajectory class included 107 (5.3%) women who had increasing P-NA symptom scores and the decreasing trajectory class included 109 (5.4%) women who had decreasing P-NA symptom scores throughout pregnancy.

Mean (SD) P-NA symptom scores of each trajectory at each trimester are shown in the footnote of Figure 1. At each assessment, the mean P-NA symptom score of the reference group was significantly lower compared with the trimester scores of the increasing and decreasing class (analysis of variance: post hoc Tukey: all $P < 0.001$). Therefore, we merged these three classes into two groups: a reference group of 1820 women with persistently low P-NA symptom scores throughout pregnancy and a group of 216 women with persistently high P-NA symptom scores throughout pregnancy. This variable was entered into a multiple logistic regression model as dependent variable with the CuZn ratio as an (continuous) independent variable, adjusting for confounders that are known to be associated with high P-NA symptom scores: parity, unplanned pregnancy, and previous diagnosis of depression as well as prepregnancy body mass index as confounder for Cu and Zn concentrations. A higher CuZn ratio was independently associated with belonging to the group with persistently high P-NA symptom scores (OR = 1.52; 95% CI, 1.13–2.04): with every 1-unit (1.0) increase of the CuZn ratio, the likelihood of having persistently high P-NA symptoms increased with 52%.

Finally, we performed a sensitivity analysis excluding women with a high P-NA score at 12 wk of gestation. Because the Tilburg Pregnancy Distress Scale–NA subscale has no cutoff, we applied a commonly used way of defining a cutoff in the psychology field (when the scores are normally distributed): a score >1 SD above the mean ($6.6 + 4.4$), which was a cutoff score of 11. At 12 wk of gestation, a total of 317 women (15.6%) scored above this cutoff and were excluded. In the remaining 1719 women, 74 had a high

CuZn level (>95 th percentile), in which a similar distribution was found as in the total group of women with a low Zn (<5 th percentile, 36.5%) or high Cu level (>95 th percentile, 40.5%). We performed another growth mixture modeling analysis with P-NA symptom scores across three time points. This resulted in a two-class solution (based on a minimum class size of 1%; Supplementary Table 2): a reference group of 1546 (89.9%) women with persistently low P-NA scores throughout pregnancy (4.5 to 4.9) and a group of 173 (10.1%) women who had an increasing P-NA symptom score toward end term (6.8, 9.8, and 11.8 at 12, 22, and 32 wk gestation, respectively). The CuZn ratio, assessed at 12 wk gestation, significantly predicted the development of increasing P-NA symptom scores (OR = 1.52; 95% CI, 1.11–2.07), also after adjustment for the same confounders (OR = 1.40; 95% CI, 1.09–1.95).

Discussion

The present prospective study found that high Cu plasma levels and a high CuZn ratio were significantly related to high CRP plasma levels, whereas low Zn plasma levels were not, assessed at 12 wk of gestation. Moreover, we found that women with a higher CuZn ratio were more likely to have persistently high P-NA symptom scores throughout pregnancy, after adjustment for confounders. Also, among women reporting low P-NA symptom scores at 12 wk of gestation, a higher CuZn ratio independently predicted the occurrence of increasing P-NA symptom scores toward end gestation.

The significant association between high Cu levels and a high CuZn ratio (>95 th percentiles) with high CRP levels has repeatedly been found in the general (non-pregnant) population [26]. Moreover, a high CuZn ratio has been associated with the increased oxidative status of ectopic pregnancy [27], the inflammatory state of children with a chronic disease [28], and proved to be a meaningful diagnostic biomarker for early-onset congenital infections [29].

In the present study, a high CuZn ratio could be attributed to low Zn levels (<5 th percentile) in $\leq 40\%$ of the cases. Also, because of hemodilution and decreased albumin levels, the Zn serum/plasma concentration declines during pregnancy and adequate Zn levels are probably lacking in $\leq 82\%$ of pregnant women worldwide [30]. Zn can have antiinflammatory effects by modulating proinflammatory T helper cells 17 and 9 that are important for adequate immune functioning in general [31,32] as well during (normal) pregnancy [33]. These characteristics might explain why a high CuZn ratio (reflecting high Cu and low Zn levels) is related to high CRP levels.

In the present study, a higher CuZn ratio in the first trimester was associated with persistently high pregnancy-specific distress symptoms throughout pregnancy. Depression and anxiety (i.e., psychological distress) have a multifactorial origin including biological, psychological, and social factors. It is an interplay of genetic factors, neurobiochemical changes, neuroendocrine axis, cytokines, and increased oxidative stress involved in the onset and progress of depression [34,35]. Several hypothetical mechanisms point to the involvement of essential metals in (subtypes of) depression: the monoamine hypothesis (including the neurotransmitters dopamine, norepinephrine, and 5-hydroxytryptamine [serotonin]) [36], brain receptor hypothesis (glutamate and corticotropin-releasing hormone) [37], neuroendocrine system hypothesis [34,38], and immune system hypothesis [39]. Cu and Zn play a role in all these suggested mechanisms [13]. A recent meta-analysis of observational studies found an association between depression and increased Cu blood levels [13]. An earlier meta-analysis found an association between lower Zn plasma levels and depression. Together, these findings might explain the highly significant

association in the present study between pregnancy-specific distress and a higher CuZn ratio [40].

The outcome of the sensitivity analysis in the present study is intriguing: in women without high pregnancy-specific distress symptoms at 12 wk of gestation, a higher CuZn ratio independently predicted the development of subsequent increasing pregnancy-specific distress symptoms throughout pregnancy. This finding indicates that in women with low distress symptoms a higher CuZn ratio in the first trimester can lead to the development of pregnancy-specific distress symptoms during pregnancy. Two prospective studies in depressed (non-pregnant) patients in which blood Cu was assessed before and after treatment found a decrease of the Cu concentration in those responding to antidepressant treatment [13]. In a recent study in mice, copper chloride treatment for 4 months was associated with the development of depressionlike behavior [41]. Hippocampal astrocytes and microglia increased after Cu exposure, suggesting that the neuroglial cells play an important role in the pathogenesis of depression, possibly by dysregulation of synaptic function and enhanced neuroinflammation [41].

A challenging explanation at a molecular level could be the link between the Zn enzyme guanosine triphosphate cyclohydrolase I and the synthesis of an enzymatic cofactor tetrahydrobiopterin, the latter being essential at central levels, for inflammation, regulation of oxidative stress, and neurotransmission including the synthesis of serotonin, dopamine, and nitric oxide [42,43]. Alterations in tetrahydrobiopterin levels—for example, because of low Zn concentration—have been documented in several brain disorders including depression, in which increased oxidative stress, inflammation, and alteration in monoaminergic function are described [43]. A combination of low Zn and high Cu levels (resulting in altered Cu containing amine oxidase enzyme activity) could contribute to increased levels of psychological distress. Recently, the possible effects of severe (psychiatric) eating disorders—including anorexia nervosa and bulimia nervosa—on nutritional status have been reviewed [44]. However, it should be noted that all these disorders were exclusion criteria in the present study.

The present study has strengths and limitations. A major strength is its large sample size. Moreover, pregnancy-specific distress symptoms were assessed prospectively and growth mixture modeling statistics enabled to distinguish women with persistently low symptom scores from those with persistently high symptom scores, overcoming the limitations of a cross-sectional design. The large sample size also enabled a sensitivity analysis in a subsample of women with low pregnancy-specific distress symptoms, discriminating women with a higher CuZn ratio at first trimester who proved to be at risk developing high pregnancy-specific distress symptoms throughout pregnancy. The present study also assessed other important determinants of pregnancy-specific distress, including a previous episode of depression and an unplanned pregnancy, but the significant association between the CuZn ratio and high pregnancy distress symptom levels persisted after adjustment for these confounders. The assessment of CRP at the same time point as assessment of Zn and Cu enabled to suggest a possible inflammatory origin of the association between high CuZn levels and high pregnancy-specific distress symptom levels. Limitations include the assessment of Zn and Cu only in the first trimester. It is a matter of speculation whether initial high Cu and low Zn levels reflect CuZn ratio throughout pregnancy. Because of hemodilution and fetal requirements of Zn, it is highly unlikely that in women with low Zn levels at first trimester, plasma levels will increase toward end of term, despite supplement intake (containing low

Zn levels). Future research should focus on trimester-specific Zn and Cu concentrations. Another limitation is the assessment of pregnancy-specific distress at a symptom rather than at a syndrome level (for obvious logistical reasons). However, the *Diagnostic and Statistical Manual of Mental Disorders* (Fifth Edition) advises that psychological distress (i.e., depression and anxiety) symptoms are assessed regarding their intensity rather than using a dichotomous definition only [45]. Also, although in the present study high CRP, an acute-phase protein, was associated with high Cu and CuZn ratio levels, the assessment of ceruloplasmin, another acute-phase protein, could have provided a more direct link between high Cu levels and increased pregnancy-specific distress symptoms.

Another limitation is that the food intake was not carefully evaluated, limiting insight into the nutritional status of the participating women. However, it should be noted that especially the first trimester of pregnancy is characterized by nausea and vomiting due to high levels of human chorionic gonadotropin, which often result in food intake characteristics that may be substantially different from a normal prepregnancy situation. We previously reported that about 25% of women during the first trimester report severe nausea and vomiting and that—apart from psychological determinants—the severity of nausea and vomiting increased with higher quartiles of chorionic gonadotropin concentration: women with chorionic gonadotropin levels in the highest quartile had the highest levels of nausea and vomiting symptoms [46]. As a consequence, a careful food intake questionnaire during the first trimester of pregnancy is less reliable because it is contaminated by episodes of (frequent) vomiting, which makes it difficult to calculate the real intake of food by the body. Also, there is general agreement that the use of food intake questionnaires may be subject to measurement errors, because they rely on memory and reported intakes, resulting in recall bias [47]. In this light, it is important to note the concept of “pregnancy brain”, referring to cognitive and memory deficits typically related to pregnancy and mostly explained by the substantial hormonal changes throughout pregnancy, which makes the use of food intake questionnaires even less reliable during pregnancy [48,49].

Another limitation might be that the prevalence of pica (the habitual ingestion of non-nutritive substances) was not evaluated. Pica has been associated with low iron intake. However, pica during pregnancy seems to be especially prevalent in black cultures in the southern states of the United States [50]; however, a Danish study (Scandinavian pregnant women are very similar to Dutch pregnant women) reported three cases in >70 000 respondents [51]. Also, there is still debate whether plasma Zn and Cu readily respond to Zn and Cu in the diet. We reported previously that during early gestation Zn and Cu plasma levels did not differ between pregnant women taking supplements or not [52]. This confirmed a similar finding in the general population of the United States [53]. Regarding the effect of extra Cu intake, a review found no effect of Cu supplementation on Cu plasma or serum levels [54]. The possible explanation is the Cu homeostasis, achieved by the coordination of the absorption efficiency in gastrointestinal tract and the excretion rate of endogenous Cu. A high-Cu diet will lead to more Cu being absorbed at the cost of increased endogenous Cu excretion whereas a low-Cu diet results in the opposite: limitation of endogenous Cu excretion and increase of Cu absorption intake [55]. Although the present study did not address the cause of the association between the CuZn ratio and pregnancy-specific psychological distress, the findings in the literature suggest that this relation might be an intrinsic response to a certain stimulus not related to diet.

Conclusions

Cu and more specifically the CuZn ratio were found important biomarkers of high pregnancy-specific distress symptom levels throughout gestation. Researchers on perinatal mental health should incorporate the (preferentially repeated) assessments of these trace elements in their study design.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2022.111938.

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