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Posted Date: 18 February 2025

doi: 10.20944/preprints202502.1318.v1

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 doi:10.20944/preprints202502.1318.v1

Article

Defining Cut-Off Scores of a High Cu/Zn Ratio in Blood Plasma During Early Pregnancy

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Abstract Background / Objectives Pregnancy is a state of increased physiological inflammation. The Cu/Zn ratio in general has been associated with inflammation. The current study evaluated a cut-off of the Cu/Zn ratio taking a high CRP as outcome variable and investigated possible associations between a high Cu/Zn ratio and obstetric complications. **Methods** Using the Receiver Operating Characteristic curve (ROCcurve) technique, an upper cut-off level of the Cu/Zn ratio was evaluated in plasma of 2035 pregnant women with a high CRP (>95th percentile) as outcome test, assessed at 12 weeks of gestation. The occurrence of a high BMI, gestational diabetes (GDM), and high psychological distress in relation to a high Cu/Zn ratio was evaluated using logistic regression analysis (OR; 95% CI). **Results** ROC analysis showed an area under the curve of 0.81 (95% CI: 0.78 – 0.84) and an upper Cu/Zn ratio cut-off level of 2.55 (sensitivity 64%, specificity 84%). Of the 382 women with a high Cu/Zn ratio, 60-66% showed adequate Cu and Zn levels (between the 10th-90th percentiles). Women with a pre-pregnancy BMI > 30 were three times more likely to present with a high Cu/Zn ratio (OR: 2.97; 95% CI: 2.15-4.13). Compared to 1304 women without any obstetric complication, the 108 women who developed GDM were two times more likely to present with a high Cu/Zn ratio at 12 weeks of gestation (O.R.: 1.9, 95% CI: 1.23 – 3.01). Also, women with a high Cu/Zn ratio were almost twice as likely to report persistently high distress symptom levels throughout gestation (O.R. = 1.9, 95% CI: 1.4-2.6). **Conclusions** A first trimester high Cu/Zn ratio of 2.55 discerns women at risk for developing GDM and high distress symptom levels throughout gestation.

Keywords: pregnancy; inflammation; oxidative stress; Cu/Zn ratio; BMI; diabetes; psychological distress

Introduction

From conception, pregnancy is a state of physiological inflammation and oxidative stress (OS) with a high metabolic rate in order to facilitate the implantation of the embryo and optimise decidualisation. [1,2] This state of arousal is also responsible for a heightened response of T-helper cells (Th1 and Th2) which is important for immune tolerance throughout pregnancy to keep the (partial) fetal allograft. [3] There is growing evidence that a balanced Th1/Th2 ratio is largely influenced by the pregnancy hormone human chorionic gonadotrophin hormone (hCG). [4]
Implantation is accompanied by the production of pro-inflammatory cytokines including interleukin-6 and -8 (IL-6 and IL-8). [5] C-reactive protein (CRP), which is synthesized in the liver, serves as an early marker of inflammation that can activate the complement system. [6] Also, OS has been closely related to inflammation markers such as IL-6 and CRP. [1,2] Under normal conditions, a balance between pro-inflammatory and anti-inflammatory responses supports trophoblast invasion and

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placental development. Uterine NK cells, macrophages, and Tregs cells interact to facilitate this process. [1,2] When these immune interactions become dysfunctional (as in infectious diseases, autoimmunity), uterine NK cells may fail to recognize and support trophoblast invasion effectively. [1,2] Additionally, an imbalance in cytokine production, with elevated levels of proinflammatory cytokines such as TNF-α and IL-6 and reduced levels of anti-inflammatory cytokines, contributes to endothelial dysfunction and impaired placentation. [5]The pro-inflammatory environment not only affects trophoblast invasion but also promotes OS, further damaging the placental tissue. [1,2] High concentrations of pro-inflammatory parameters have repeatedly been associated with the 'big four' obstetric complications: preterm birth, pre-eclampsia, gestational diabetes, and intra-uterine growth retardation. [7]

During the last decades it has become apparent that several metal ions as essential nutrients have roles in the context of infection, mental health, immune function, and inflammatory responses during pregnancy and that these entities are interconnected. [8] *Zinc* (Zn) serves as a cofactor in at least 3000 human proteins and can express anti-inflammatory and antioxidant characteristics that benefit numerous normal metabolic and brain functions. [9] In general, Zn supplementation lowers human C-reactive protein (CRP) levels in pregnant women. [10] *Copper* (Cu) serves as a cofactor in redox and oxygen chemistry. [11] High levels of Cu are associated with high levels of ceruloplasmin, an acute phase protein, modifying the concentration of pro-inflammatory cytokines (IL-1, IL-6) and resulting in increased CRP production by the liver. [12] Copper exposure can also perturb brain inflammatory responses. [13] In general, an excess of Cu exhibits pro-oxidant activities and increases OS by generating free radicals, while Zn can compete with Cu in the formation of such reactive and damaging species. Cu and Zn absorption from food are also interconnected: high Zn levels will result in lower absorption of Cu from the intestines, and vice versa: high Cu levels will impair Zn absorption. [11] In the blood (plasma and serum), copper and zinc concentrations are regulated and therefore remain at a ratio of 1:1. [11] However, in inflammation, this Cu/Zn ratio is perturbed: In an acute phase response Cu increases in blood while Zn decreases. [11] Thus, the Cu/Zn ratio combines alterations of both micronutrients and may constitute a meaningful diagnostic blood biomarker for psychological and metabolic distress. In adult populations, several studies have shown that the serum Cu/Zn ratio is a better predictor of disease severity and/or mortality than Cu levels alone. [14] Also, a higher serum Cu/Zn ratio was associated with cancer diagnosis, prognosis, tumor stage, and overall survival of cancer patients, as well as overall mortality, mortality from cardiovascular disease and infection. [15]

There is growing evidence that the BMI status at the higher spectrum (obesity) can be regarded as a chronic state of low-grade inflammation. [16] Individuals that are overweight and obese have altered serum levels of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP) and interleukins (IL-6, IL-8). [16] This implicates that, if Cu, Zn and/or the CuZn ratio are related to high CRP levels, BMI status also will be related to the Cu/Zn ratio.

Perturbations of Zn and Cu metabolism have also been associated with diabetes in general and with gestational diabetes (GDM) more specifically. [17,18] The weighted GDM prevalence between 2014-2019 was highest in the Eastern Europe (31.5%), followed by Southern Europe (12.3%), Western Europe (10.7%, and Northern Europe (8.9%). [19] Zn is vital for the appropriate processing, storage, secretion and action of insulin [9]. Conversely, diabetes affects Zn homeostasis and is responsible for increased urinary loss resulting in decreased total body zinc [9]. A recent review showed that diabetics in general have higher blood Cu levels compared to non-diabetic controls [20,21]. During a "normal" pregnancy, the serum Cu concentration increases by 30% to meet the requirements of Cu-containing enzymes in the maternal part as well as the increasing fetal demands. [12] The serum Cu concentrations are remarkably increased in GDM patients compared to healthy pregnant women. [21] GDM is related to poor fetal prognosis and obstetric complications and – during the last decades – became more prevalent at the population level.

Psychological distress is highly prevalent in perinatal women since up to 20% of pregnant women report high levels of distress symptoms [22] and it can be defined as "a state of emotional

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suffering characterized by symptoms of depression and anxiety sometimes accompanied by somatic symptoms" [23]. Psychological distress during pregnancy has also been associated with impaired obstetric outcome including intrauterine growth retardation, preterm birth, and low birth weight [24]. We previously demonstrated an independent association between the Cu/Zn ratio levels, assessed at 12 weeks gestation, and the subsequent development of pregnancy distress symptoms throughout gestation [25].

The evaluation of qualifiable and quantitative pathophysiological alteration related to psychological and metabolic (GDM) distress may benefit future outcomes for both mother and infant. In this regard, the serum Cu/Zn ratio may be an important clinical parameter. However, for clinical implications, one would need to define an appropriate cut-off for the Cu/Zn ratio. Therefore, the current study evaluated a cut-off for the Cu/Zn ratio in a large birth cohort of over 2000 pregnant women, using a high CRP level, reflecting increased inflammation, as reference. Subsequently, the nature of the Cu/Zn ratio above the cut-off was evaluated (high Cu and / or low Zn levels?). Finally, the possible associations between high Cu/Zn ratios and several clinical conditions were evaluated: high BMI (obesity), the occurrence of GDM, and high distress symptom levels during pregnancy.

Materials and Methods

The recruitment of pregnant women has been described in detail elsewhere [26]. In sum, during a recruitment period of 18 months, approximately 4,150 women visited the participating midwife offices. Only Dutch speaking women (N=3,475) were invited to participate. 3,159 Women were eligible of whom 2,275 (response rate = 72%) signed a written informed consent.[26] The study was completed in accordance with the Declaration of Helsinki as revised in 2013 and was approved by the Psychology Ethics Committee of Tilburg University (protocol number EC-2012.25) and additionally evaluated by the Medical Ethical Committee of the Máxima Medical Center in Veldhoven. In total, 2035 completed all questionnaires and had all biochemical assessments. Therefore, these women were included for data-analysis.

Assessments

This study is reported in line with the STROBE guidelines. At 12 weeks of gestation, women completed a set of questionnaires that evaluated demographic and obstetric features and life-style habits. During pregnancy, depression (Edinburgh Postnatal Depression Scale, EPDS) and distress symptoms (Tilburg Pregnancy Distress Scale, TPDS) were assessed at 12, 22 and 32 weeks of gestation. [27,28] A research midwife evaluated all birth record forms including possible obstetric complications.

Copper and Zinc

Copper (Cu) and zinc (Zn) were assessed in 12-week heparin plasma samples by dynamic reaction cell inductively coupled plasma mass spectrometry (DRC ICP-MS) using a Nexion 300X (Perkin-Elmer, Groningen, The Netherlands) in the kinetic energy discrimination (KED) mode. Helium (at 1.0 mL/min) was employed as the KED gas to remove polyatomic interferences. Within- and between-run variation was assessed by CLSI guideline EP5-A2 and found to be less than 3% for low and high concentrations of Cu and Zn in plasma. According to CLSI guideline EP 17-A2, the lower limit of quantitation was calculated at 0.27 and 0.30 µmol/L for Cu and Zn, respectively. Certified reference materials Seronorm L1 or L2 (Nycomed, Norway) were used to continuously monitor accuracy. During measurement of the samples of this study, values for L1 (lot 1309438) for Cu and Zn were 17.9 µmol/L (reported analytical value 17.1 µmol/L and range 15.7 – 18.5 µmol/L) and 17.4 µmol/L (reported analytical value 16.8 µmol/L and range 14.6 – 19.0 µmol/L), respectively. For L2 (lot 1309416) values for Cu and Zn were 29.4 µmol/L (reported certified mean 29.1 µmol/L and range 26.7 – 31.5 µmol/L) and 25.7 µmol/L (reported certified mean 24.7 µmol/L range 21.5 – 28.0 µmol/L), respectively. Moreover, pooled plasma was measured as well to monitor within-run

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variation at low and high concentrations.

An important methodological issue, when defining adequate Cu and Zn levels is the definition of a reference group. It makes little sense to define too low levels using a cut-off < 2.5th or < 5th percentiles and to regard women with, for example, a 3rd or 6th percentile level, respectively, as belonging to the normal group. Thus, we feel that the reference group should refer to women with not too low nor too high Cu and Zn concentrations, for example between the 10th - 90th percentiles. It is reasonable to accept that these 80% of women reflect adequate levels of Cu and Zn. Therefore we defined a reference group consisting of women with definitely adequate Cu or Zn levels using the 10th – 90th percentiles as reference ranges. Too low or too high Cu and Zn concentrations were defined as concentrations below the 10th and above the 90th percentiles, respectively. This approach has proven to be clinically relevant in thyroid research. [29]

C-Reactive Protein (CRP)

CRP (not high sensitive CRP) was measured in 12 week Li-heparin plasma samples using a immunoturbidimetric assay (Cobas® c702 platform; Roche Diagnostics, Mannheim, Germany). The lower limit of quantification was 0.6 mg/L. Within-laboratory coefficients of variation were 2.5% at 7.0 mg/L and 2.2% at 51.5 mg/L. According to the guidelines of the international Clinical Chemistry Society, because CRP has only one abnormal cut-off at the upper level (a too low CRP value is not defined), a concentration above the 95th percentile was used to define a high CRP level. [30]

Statistics

Statistical analysis was performed using the IBM SPSS Statistics for Windows v 28.0 (IBM Corp., Armonk, NY). Descriptive statistics were used to analyze Cu and Zn levels and to calculate the Cu/Zn ratio. Correlations between Cu, Zn and the Cu/Zn ratio versus CRP were calculated. We used Receiver Operating Characteristic curve (ROC-curve) technique to detect a possible upper cut-off level of the Cu/Zn ratio, using high CRP as outcome test. ROC analysis is a powerful tool for assessing the diagnostic performance of index tests, which are tests that are used to diagnose a disease or condition. [31] The AUC value is a summary metric of the ROC curve that reflects the test's ability to distinguish between diseased and non-diseased individuals. AUC values range from 0.5 to 1.0, with a value of 0.5 indicating that the test is no better than chance at distinguishing between diseased and non-diseased individuals. A value of 1.0 indicates perfect discrimination. AUC values above 0.80 are generally considered clinically useful, while values below 0.80 are considered of limited clinical utility. [31] ROC analysis also facilitates the identification of an optimal cutoff value, particularly when the AUC value surpasses 0.80, by presenting a coordinate table showing different combinations of sensitivity and false positive (1-specificity) at different cut-offs. The threshold value that maximizes both sensitivity and specificity defines the cut-off. [31] Subsequently, we evaluated the Cu and Zn characteristics of the women with a Cu/Zn ratio above this cut-off. Finally, we performed logistic regression (O.R.; 95% CI) to evaluate a possible association between a Cu/Zn ratio above the cut-off and the occurrence of obesity and GDM and between a high Cu/Zn ratio and women with high levels of distress symptoms during gestation.

Results

Table 1 shows the characteristics of 2035women with all biological parameters assessed at 12 weeks of gestation.

Table 1. Characteristics of 2,035 women with Zn, Cu, and CRP assessed at 12 weeks of gestation.

	Mean (SD)	N (%)	median / range
Demographic features			

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Caucasian		2015 (99)
Age (in years)	30.5 (3.5)	
Educational level		
Low		576 (28.3)
Medium		114 (5.6)
High*		1345 (66.1)
Marital status		
With partner		1925 (94.6)
Single		110 (5.4)
Obstetric features		
Primipara's		999 (49.1)
Multipara's		1036 (51.9)
Previous miscarriage		507 (24.9)
Lifestyle habits during pregnancy		
Smoking		126 (6.2)
Any alcohol intake		73 (3.6)
Pre-pregnancy BMI	23.8 (3.7)	
BMI < 18.5		66 (3.2)
BMI 18.5 – 25		1319 (64.8)
BMI 25 – 30		470 (23.1)
BMI 30 – 35		142 (7.0)
BMI > 35		38 (1.9)
Biological parameters		
Zinc µmol/L	12.56 (1.80)	12.44 (7.51 – 20.94)
Copper µmol/L	26.26 (4.74)	25.9 (12.85 – 47.84)
Cu / Zn ratio	2.13 (0.5)	2.09 (0.98 – 4.0)
CRP mg / L		3.7 (0.11 - 49)
CRP>95thpercentile (>15mg/L)		110 (5.4)

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[a]Bachelor's or Master's degree. BMI, body mass index.

Almost two thirds of the women were highly educated. Using the WHO classification BMI system, the percentage of obese women (BMI > 30) was 8.8 %.

Cu, Zn and Cu/Zn ratio figures were normally distributed while CRP figures were not. The (Spearman) correlations between CRP and Cu, Zn and the Cu/Zn ratio were: 0.50 ($P < 0.001$), - 0.04 ($P = 0.08$), and 0.45 ($P<0.001$), respectively. According to the area under the curve (AUC) on the receiver operating characteristic (ROC) curve for the prediction of high CRP, the Cu/Zn ratio (AUC 0.81, 95% CI 0.78-0.84) proved to be an appropriate predictor. The coordinates table of the curve showed that a Cu/Zn ratio cut-off of 2.55 detected high CRP with the combination of highest sensitivity (64%) and lowest false positive (1 – specificity, 16%) figures. There were 382 (18.8%) women with a Cu/Zn ratio above this cut-off. These women had significantly more often a high CRP level: $X2 (1) = 153$, $P < 0.001$ (large effect size). A closer look at the two different cells of the chi-square test (df=1) between high CRP and a high Cu/Zn ratio showed that the standardized residual of the high CuZn group was 2.6. This means that women in this group had a high CRP significantly more frequently than would be expected if the Cu/Zn ratio were not related to CRP. [32]

We subsequently looked at the characteristics of the 382 women with a high Cu/Zn ratio using this cut-off of 2.55 with regard to their Cu and Zn concentrations (Figure 1).

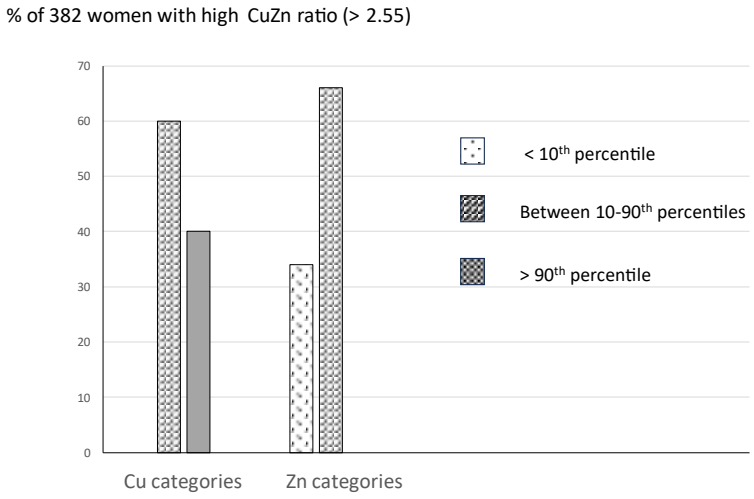

Figure 1. Distribution of Cu and Zn sub-categories in 382 women with a Cu/Zn ratio above cut-off (> 2.55).

The 10th and 90th percentiles of Cu and Zn were 10.4 µmol/L and 20.4 µmol/L, and 14.9 µmol/L and 32.5 µmol/L, respectively. Up to 40% of the women with a Cu/Zn ratio above the cut-off showed a Cu concentration above the 90th percentile while 60% had adequate (between 10-90th percentiles) Cu levels (no women showed Cu levels < 10th percentile). Up to one third of the women with high a Cu/Zn ratio showed a Zn concentration below the 10th percentile, the remaining 66% women had adequate Zn levels (no women showed Zn levels above the 90th percentile). Thus, 60-66% of the high Cu/Zn ratios could not be contributed by either Cu or Zn levels in the too high or too low range, respectively.

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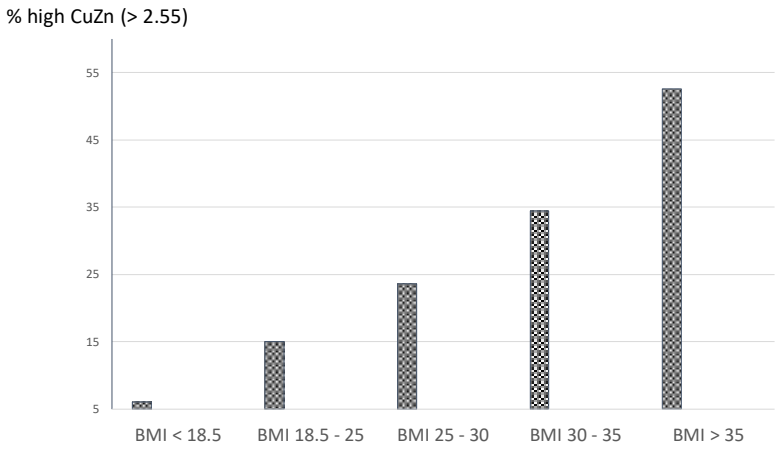

Figure 2. Percentage of high Cu/Zn ratios (> 2.55 according to BMI classes).

In Figure 2, the percentages of a high Cu/Zn ratio cut-off women according to BMI classification are shown. With increasing BMI class, the percentage of women with a Cu/Zn ratio above the cut-off increased significantly: from 6.1% in class 1 (underweight) to 52.6% in class 5 (obese), X2 (4): 78, P < 0.001. The standardized residuals in the five different cells were: - 2.4, varied from – 3.2, 2.4, 4.3, 4.8, respectively, suggesting that women in these subgroups had a low or high BMI significantly more frequently than would be expected if the Cu/Zn ratio were not related to the BMI status. [32] Women with a pre-pregnancy BMI > 30 were three times more likely to present Cu/Zn ratios above the cut-off: O.R.: 2.97 (95% CI: 2.15-4.13).

There were 108 (5.3%) women who developed GDM. In order to evaluate the possible association with a high Cu/Zn ratio, we first defined a group of healthy women without any obstetric complication: N = 1304 (64%). The remaining 623 (30.6%) women showed other complications such as pre-eclampsia, intra-uterine growth retardation, or pre-term birth or had a non-spontaneous birth for other reasons. Some of these conditions have also been related to high Cu or low Zn concentrations [8]. Comparing the 108 GDM women with the 1304 women in the healthy group, women with a high Cu/Zn ratio were almost two times more likely to develop GDM: O.R.: 1.9, 95% CI: 1.23 – 3.01, p = 0.004.

Finally, we previously demonstrated – using Latent Class Growth Analysis – an independent association between a higher Cu/Zn ratio and persistently high pregnancy distress symptoms throughout gestation using a much higher Cu/Zn ratio cut-off: > 3.0 μmol/L, corresponding with the 95th Cu/Zn ratio percentile. [25] In the current sample, there were 1781 women with persistently low distress symptoms and 254 (12.5%) women with persistently high distress symptoms throughout pregnancy assessed on the TPDS. Women with a high Cu/Zn ratio (> 2.55) were almost two times more likely (O.R. = 1.9, 95% CI: 1.4-2.6) to report persistently high distress symptom levels throughout gestation. When we excluded women with high depressive symptoms (EPDS scores >10, n = 248) at 12 weeks (a major risk factor of high levels of distress symptoms), in the remaining 1787 women, those with a high Cu/Zn ratio were 2.1 (CI: 1.48 – 3.0) times more likely to present persistently high distress symptoms. Thus, in women with low depression symptom levels, a Cu/Zn ratio cut-off of 2.55 predicted the occurrence of persistently high distress symptoms throughout gestation.

Discussion

The current study defines a high Cu/Zn ratio based on the association with high CRP levels. A cut-off of 2.55 showed adequate sensitivity and specificity predicting high CRP (> 95th percentile) levels. Moreover, this cut-off showed clinical relevance in its association with pre-pregnancy BMI

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classes, in detecting women during early gestation who subsequently developed GDM as well as women who reported persistently high distress symptom levels throughout pregnancy.

Both zinc and copper can have anti- or prooxidant activities depending on their concentrations. [8] Zinc is required for the function of over 300 enzymes and 1000 transcription factors and promotes antioxidant activity. [9] Lower Zn levels will result in reduced antioxidant capacity increasing the risk of oxidative stress and inflammation. [8] Copper can catalyse the formation of free radicals, including undesirable hydroxyl radicals, and thus at higher cellular levels contributes to increased oxidative stress. [8] Acute infections result in an increase of the serum copper and a decrease of serum zinc due to redistribution through the activation of inflammatory cytokines (such as IL-6) in the liver triggering an acute phase reaction [33,34]. High IL-6 concentrations will increase CRP production by the liver. For example, the Cu/Zn ratio has been proven to be diagnostic in several infectious diseases, including giardiasis, amoebiasis, tuberculosis, malaria and COVID-19 [33,34]. Therefore, there is sufficient pathophysiological evidence to correlate Cu/Zn ratio levels to CRP concentrations. A further exploration of the Cu and Zn concentrations constituting the ratio showed that almost up to two thirds of the Cu/Zn ratio above the cut-off (> 2.55) does not apply to women with very high Cu or very low Zn concentrations. Remarkably, the majority of women with a Cu/Zn ratio above the cut-off consisted of a combination of Cu and Zn levels in the fairly normal range: between the 10-90th percentiles while the Cu/Zn ratio is not in the normal range. Therefore, the Cu/Zn ratio provides an independent clinical measure of endogenous perturbation through inflammation and could be used to detect women at risk for pregnancy complications, rather than using Cu and Zn levels separately. Higher levels of BMI have been associated repeatedly with increased levels of inflammation markers, including CRP. [16] The association between a high Cu/Zn ratio and high CRP explains the strong association between obesity and a high Cu/Zn ratio. Obesity increases the concentration of inflammation markers resulting in oxidative stress which will increase Cu and decrease Zn levels in serum. [16] Zn levels have been associated directly with insulin functioning and high Cu levels can lead to insulin resistance through OS. [9] It is well known that pre-pregnancy obesity is by far the most important predictor of GDM. [19] For example, in the current study – within the group of women with GDM (N=108) and healthy controls (N = 1304) – there were 108 women (7.7%) with a pre-pregnancy BMI > 30. These women were five times more likely to develop GDM (O.R.: 5.3, 95% CI = 3.2 – 8.6). The associations between a high Cu/Zn ratio and CRP, high BMI and GDM, high BMI and high CRP, suggest that the association between a high Cu/Zn ratio and GDM is most likely BMI-mediated. Also, BMI and OS are significantly positively correlated [16].

We previously described an association between persistently high distress scores and Cu/Zn ratios [25] However, in that study a high Cu/Zn ratio was defined using a 95th percentile cut-off (3.05). The current study shows, after ROC statistics, that an even lower cut-off (>2.55), corresponding to the 81st percentile, is already associated with persistently higher levels of distress symptoms. Also, applying a cut-off of 3.05 in the current study would predict high CRP with a sensitivity of 22% and a specificity of 95% (ROC statistics). In a very recent meta-analysis, the blood Zn and Cu levels were compared between depressed patients and healthy controls. [35] Zn levels were significantly lower in 1364 depressed patients compared to 1246 healthy controls, while Cu levels were significantly higher in 1883 depressed patients compared to 1455 healthy controls. Also, significantly higher serum Zn levels were demonstrated after using antidepressant SSRIs while serum Cu levels significantly decreased compared to pre-treatment condition. [36]

Thus, the Cu/Zn ratio combines alterations of both micronutrients and may constitute a meaningful diagnostic biomarker for psychological distress, even at the low cut-off defined in this study. The 2.55 Cu/Zn ratio cut-off – representing the 81th percentile – in the current study is much higher than the 97.5th plasma cut-off of 1.65 of a study in China in 191 women of childbearing age (18-45 years). [37] Assuming that pregnant women did not participate (pregnancy was not mentioned as an exclusion criteria), a possible explanation could be that plasma copper concentrations in general increase during pregnancy by 30% resulting in a higher Cu/Zn ratio cut-off in the current study. [12]

The current study has several strengths and limitations. To our knowledge, it is the first report of the determination of a Cu/Zn cut-off as an inflammatory marker. A major strength is the sample

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size enabling sufficient power for statistical analyses. The current sampling is representative of the total obstetric population in The Netherlands in terms of parity, mean age of pregnant women, and previous abortion figures (National Data on Dutch Birth Outcome, Perined). [38] Moreover, the study combined biological data with several highly clinically relevant conditions including high BMI, GDM and psychological distress symptoms that are all related to poor obstetric outcome. A major limitation is the cross-sectional assessment of Cu and Zn and the possible limitation whether maternal plasma concentrations would be associated with placental or fetal values. A recent study showed significant correlations between maternal Cu and Zn concentrations in plasma and concentrations of Cu and Zn in maternal urine and placental tissue. [39] Thus, single assessments of micronutrients in maternal plasma actually correlate urinary and placental concentrations. This further supports the use of the Cu/Zn ratio to detect women at risk for developing obstetric problems. Also, in the current study no dietary intake of Cu and Zn was assessed. Another limitation is that the women in our cohort are predominantly white Caucasian, almost all of them had a partner, up to 2/3 were highly educated and the number of women who smoked or drank alcohol and who had a BMI > 30 was lower than in the general Dutch population. [40] This hampers the generalizability of the current findings to the general population.

In conclusion, a cut-off of 2.55 defining a high Cu/Zn ratio assessed at first pregnancy trimester seems to be a useful tool to detect women at risk for developing GDM and persistently high levels of psychological distress symptoms. The fact that almost two thirds of women above this cut-off have adequate copper and zinc levels shows how incredibly tight the control of the ratio must be and how sensitive a deviation is as an important marker giving information that does not show when either zinc or copper alone seem normal.

Author Contributions: Conceptualization, V.P., W.M., and J.K.; Methodology, V.P. and J.K; Validation: J.K.; Formal Analysis, V.P.; Investigation, V.P. and J.K.; Resources, V.P.; Data Curation, V.P; Writing: V.P, W.M., and J.K. All authors have approved the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Funding: The current study was made possible by a grant of ''de Weijershorst'' Foundation.

Acknowledgments: Roche Diagnostics kindly supported the project by supplying the diagnostic kits.

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