Journal of Trace Elements in Medicine and Biology 46 (2018) 103–109

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

Clinical studies

Early pregnancy maternal trace mineral status and the association with adverse pregnancy outcome in a cohort of Australian women

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ARTICLE INFO

Keywords:
Pregnancy
Micronutrients
Copper
Zinc
Pregnancy complications

ABSTRACT

Maternal micronutrient deficiencies in pregnancy can have profound effects on fetal development and pregnancy outcome. Plasma trace minerals including copper, zinc, selenium and iron have been shown to be extremely important in supporting reproduction. We sought to determine whether there is an association between maternal trace mineral status in early pregnancy and pregnancy complications using a prospective cohort study of 1065 pregnant Australian women who were recruited as part of the Screening for Pregnancy Endpoints (SCOPE) study in Adelaide. Copper, zinc, selenium and iron present in the plasma were measured using mass spectrometry in samples collected at 15 ± 1 weeks' gestation. After adjusting for covariates, women with lower plasma copper (< 27.9 μmol/L and 27.9–32.5 μmol/L) had decreased risk for any pregnancy complication when compared with women with high plasma copper (> 32.5 μmol/L) (aRR = 0.87; 95% CI = 0.76, 0.99 and aRR = 0.88; 95% CI = 0.78, 1.00, respectively). This was also observed when adjusting for plasma zinc and selenium status (< 27.9 μmol/L: aRR = 0.81; 95% CI = 0.69, 0.96 and 27.9–32.5 μmol/L: aRR = 0.84; 95% CI = 0.72, 0.98). Combined low copper and zinc status was also associated with a reduced risk of any pregnancy complication as compared with high copper and zinc status (aRR = 0.80; 95% CI = 0.70, 0.93). These results provide justification for further work into elucidating the mechanistic role of trace elements in early pregnancy, as well as their interactions in supporting successful pregnancy outcomes.

Maternal nutrient stores and diet supply all the macro- and micronutrients to support optimal fetal growth essential for successful pregnancy [1]. Hence, it is not surprising that maternal deficiencies in key micronutrients can have profound effects on fetal development and pregnancy outcome [2]. Pregnancy complications including pre-eclampsia (PE), gestational diabetes mellitus (GDM), spontaneous preterm birth (sPTB) and fetal growth restriction (FGR) together affect 25% of first pregnancies and predict lifelong morbidity and mortality for both the mother and infant [3]. Furthermore, micronutrient deficiencies which tend to be associated with decreased consumption of foods rich in micronutrients, have also been associated with the development of PE, GDM, sPTB, FGR, as well as gestational hypertension (GH) [2].

Extensive investigations into micronutrient deficiencies have focused on those common within pregnant populations including folate and vitamin D [4]. However, evidence is emerging about the importance of trace minerals like iron, zinc and copper in supporting successful pregnancy [5]. It is known that trace minerals are crucial for the maintenance of cell proliferation and function with severe deficiencies in copper and zinc during pregnancy having been shown to have a teratogenic effect on the fetus [6]. This is likely driven by a reduction in the activity of key enzymes which require these metals structurally in order to function, as well as compromised oxidant defence systems [6]. It is also important to acknowledge the importance of micronutrients in mediating inflammation and the immune response. Animal models of iron, copper and zinc deficiencies have been shown to be associated with compromised immunity and increased susceptibility to infection [7]. Pregnancy complications including PE and FGR have been associated with increased oxidative stress and circulating markers of inflammation [8,9] and therefore there may be a causal connection

Abbreviations: FGR, fetal growth restriction; GDM, gestational diabetes mellitus; GH, gestational hypertension; PE, preeclampsia; sPTB, spontaneous preterm birth; SGA, small-for-gestational age

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https://doi.org/10.1016/j.jtemb.2017.11.016
Received 18 September 2017; Received in revised form 20 November 2017; Accepted 30 November 2017

between micronutrient deficiencies in pregnancy and the development of pregnancy complications mediated by oxidative stress and inflammation.

The association between trace minerals and pregnancy have been described previously [10–17], however many of these studies have been conducted in late pregnancy or at term. Given that many of these pregnancy complications originate in early gestation, it is important to also understand how micronutrient status in the first trimester is associated with adverse pregnancy outcomes. Marginal micronutrient deficiencies early in pregnancy may lead to more severe deficiencies later in pregnancy due to increased metabolic demands from the rapidly growing placenta and fetus. Thus we aimed to determine whether deficiencies in the trace minerals copper, zinc, selenium and iron at 15 ± 1 weeks' gestation may be associated with a number of pregnancy complications. As copper and zinc share similar electro-chemical properties and biological pathways [18], we also explored interactions between zinc and copper status in early pregnancy and their relationship with adverse pregnancy outcomes with the goal of better understanding how these minerals may be important to successful pregnancy.

1. Materials and methods

1.1. Study participants

Plasma samples were obtained from Adelaide participants recruited as part of the international prospective Screening for Pregnancy Endpoints (SCOPE) study. Nulliparous women carrying a singleton pregnancy were recruited at 15 ± 1 weeks' gestation from the Lyell McEwin Hospital, Adelaide, Australia between November 2004 and September 2008. Ethics approval was gained from the University of Adelaide ethics committee and all women provided written consent (approval no: REC 1712/5/2008). At recruitment, women were interviewed by a research midwife and asked questions on maternal demographics and had physical measurements recorded. These included age, body mass index (BMI) and smoking status [19]. Biochemical markers were also measured at 15 ± 1 weeks' gestation and included plasma C-reactive protein (CRP) [20]. Women were not eligible to participate in the study if they suffered from a pre-existing medical condition or had obstetric history which placed them at high risk of developing PE, sPTB or delivering a small-for-gestational age (SGA) infant. Those who had suffered three or more miscarriages or had undergone three or more pregnancy terminations were also excluded.

Uncomplicated pregnancies were defined as those without any pregnancy disorder and included normotensive women who delivered an appropriate weight for gestational age infant at term (≥37 weeks' gestation) [21]. GH was diagnosed as the development of high blood pressure (systolic blood press ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) on at least two occasions after 20 weeks' gestation. PE was defined as GH in conjunction with proteinuria (24 h urinary protein ≥300 mg or a spot urine protein:creatinine ratio ≥30 mg/mmol creatinine or urine dipstick protein ≥2) or any multisystem complication of PE or uteroplacental dysfunction [22]. GDM was diagnosed according to the International Associations of Diabetes and Pregnancy Study Groups criteria [23]. sPTB was defined as the spontaneous onset of labour at < 37 weeks' gestation. SGA was defined as birth weight below the 10th customised centile adjusted for maternal height, booking weight, ethnicity, delivery gestation and infant sex.

1.2. Elemental analysis

Venous blood samples were provided at 15 ± 1 weeks' gestation and collected into heparinised tubes in order to obtain plasma samples and then analysed using inductively-coupled plasma mass spectrometry (ICP-MS) (Agilent 7700 ICP-MS) (Agilent 5100 ICP-OES; CSIRO Analytical Services, South Australia) to measure the concentrations of copper, selenium, iron and zinc. Prior to analysis, 250 μL of plasma was digested in concentrated nitric acid (0% HNO_3) in sealed Teflon containers for approximately 48 h and then diluted. Samples were run alongside two internal standards: iridium and rhodium (Choice Analytical) at a concentration of 200 ppb and an 8-point calibration, including blank, was carried out between 0.01 μg/L and 100 μg/L. There were 47 (4.4%) women with plasma iron levels below the detection limit of 7.16 μmol/L and were therefore assigned 7.15 μmol/L.

1.3. Statistical analysis

All statistical analysis was performed in R (v3.1.1) [24]. Baseline characteristics were tested for normality using the Shapiro-Wilk test and summarised according to pregnancy outcome. Fisher's exact tests were performed for categorical variables and Welch's *t* test for continuous variables comparing women with each pregnancy complication to all other women. Plasma copper, zinc, selenium and iron were expressed as mean (± standard deviation: SD) and compared between women who developed a pregnancy complication and those who did not using a non-parametric Mann-Whitney *U*. Spearman's correlations were used to examine the relationship between each of the trace minerals with each other, as well as with circulating CRP; a marker of inflammation.

In order to assess the effects of each trace mineral on pregnancy outcome, plasma copper, zinc, selenium and iron were divided into tertiles based on their distribution amongst all women in this study. Relative risks (RR) and 95% confidence intervals (CIs) of pregnancy complications from any complication, PE, GH, GDM, sPTB and SGA for copper, zinc, selenium and iron were examined using multivariable Poisson regression with robust variance estimation. Multivariable adjustment was made for maternal age, maternal BMI and smoking status at 15 ± 1 weeks' gestation (yes compared to no) as covariates. Maternal socioeconomic status, determined by assigning the New Zealand socioeconomic index score (SEI) [25], was initially included in the adjusted model but did not change any effects observed and was subsequently removed in the final analyses. The final analyses were also repeated for copper, zinc and selenium adjusting for each other as well as covariates previously mentioned.

2. Results

Of the 1165 SCOPE women recruited in Adelaide, 1065 (91%) plasma samples from 15 ± 1 weeks' gestation were available for analysis of trace minerals. These included 558 (52%) women whose pregnancies were uncomplicated, 85 (8%) who later developed PE, 108 (10%) who were diagnosed with GH, 51 (5%) who were diagnosed with GDM, 65 (6%) who delivered spontaneously preterm and 134 (13%) who delivered an SGA infant. Mean maternal age and BMI for all women whose plasma was analysed was 23.71 ± 5 years and 27.01 ± 6.52 kg/m^2 (Table 1). Compared to those whose pregnancies were uncomplicated, women who went on to have a pregnancy complication had a higher BMI in early pregnancy but there was no difference in maternal age, smoking status or use of supplements at 15 ± 1 weeks' gestation.

Plasma trace minerals in all women ranged from 10.3 to 52.99 μmol/L for copper, 3.24–34.70 μmol/L for zinc, 0.253–1.785 μmol/L for selenium and 7.14–72.60 μg/L for iron. Mean plasma copper at 15 ± 1 weeks' gestation was higher in women who went onto have a pregnancy complication when compared to those whose pregnancies remained uncomplicated (Table 1; $P < 0.001$). Moderate differences in circulating levels of zinc, selenium and iron were also observed in the women who later developed a pregnancy complication compared to those who did not (Table 1). Circulating copper was positively correlated with both zinc (Supplementary Fig. 1A; $R^2 = 0.263$) and selenium (Supplementary Fig. 1B; $R^2 = 0.303$) but negatively correlated with iron (Supplementary Fig. 1C; $R^2 = -0.156$). Iron on the other hand, was positively

Table 1
Participant characteristics of the Adelaide SCOPE cohort in which plasma trace minerals were measured at 15 ± 1 weeks' gestation.

	All (n = 1065)	Uncomplicated (n = 558)	Complicated (n = 507)	P value
Age *yrs*, mean (SD)	23.71 (5)	23.59 (5)	23.85 (5)	0.399
(range)	[14–43]	[14–40]	[15–43]	
BMI kg/m^2, mean (SD)	27.01 (6.52)	26.30 (5.86)	27.77 (7.12)	**0.000**
(range)	[16.2–58.5]	[16.2–49.7]	[16.4–58.5]	
Smoking status, *n* (%)				0.063
No	651 (61)	352 (54)	299 (46)	
Quit	152 (14)	85 (56)	67 (47)	
Smoking	262 (25)	121 (46)	141 (54)	
Education, *n* (%)				0.410
No Secondary	422 (40)	211 (50)	211 (50)	
Secondary	608 (57)	327 (54)	381 (46)	
Tertiary	35 (3)	20 (57)	15 (43)	
Supplement Use, *n* (%)				0.535
No	105 (10)	51 (49)	53 (51)	
Yes	960 (90)	506 (53)	454 (47)	
Plasma Cu *μmol/L*, mean (SD)	30.30 (5.49)	29.61 (5.18)	31.07 (5.72)	**0.000**
[range]	[10.30–52.99]	[14.76–47.73]	52.99	
Plasma Zn *μmol/L*, mean (SD)	9.39 (2.32)	9.28 (2.11)	9.51 (2.52)	0.077
[range]	[3.24–34.70]	[4.83–21.36]	34.70	
Plasma Se *μmol/L*, mean (SD)	0.919 (0.151)	0.911 (0.140)	0.929 (0.161)	0.055
[range]	[0.253–1.785]	[0.544–1.380]	[0.253–1.785]	
Plasma Fe *μmol/L*, mean (SD)	19.06 (7.77)	19.49 (7.90)	18.61 (7.61)	0.053
[range]	[7.14–72.60]	[7.14–71.06]	[7.14–72.60]	

Supplement use was defined as using any form of mineral or vitamin supplement at 15 ± 1 weeks' gestation.
P values for continuous variables were determined using Welch *t*-test, categorical variables a Fisher's exact test and plasma trace elements a Mann-Witney *U* test comparing women with a pregnancy complication to those whose pregnancies remained uncomplicated. BMI: body mass index; Cu: Copper; Zn: Zinc; Se: Selenium; Fe: Iron.

correlated with zinc (Supplementary Fig. 1E; R^2 = 0.107) and selenium (Supplementary Fig. 1F; R^2 = 0.204).

A significant positive correlation between plasma copper and plasma CRP was observed at 15 ± 1 weeks' gestation (Fig.1A : R^2 = 0.424, P < 0.001) while there was an inverse, albeit not as strong, correlation between plasma zinc and plasma iron and CRP (Fig. 1B: R^2 = −0.066, P = 0.048 and Fig. 1D: R^2 = −0.280, P < 0.001, respectively). When BMI was categorised as underweight (≤20 kg/m^2), normal weight (20.1–24.9 kg/m^2), overweight (25–29.9 kg/m^2) and obese (≥30 kg/m^2), compared with normal weight women, plasma zinc, selenium and iron was lower in the obese women (Fig. 2). Plasma copper on the other hand was significantly higher in the obese women when compared to underweight, normal weight and overweight women (Fig. 2).

The adjusted relative risks (aRR) for each pregnancy complication of plasma copper, zinc, selenium and iron categories based on population tertiles are shown in Table 2. Women with lower plasma copper (1 st and 2nd tertile) had decreased risk for any pregnancy complication

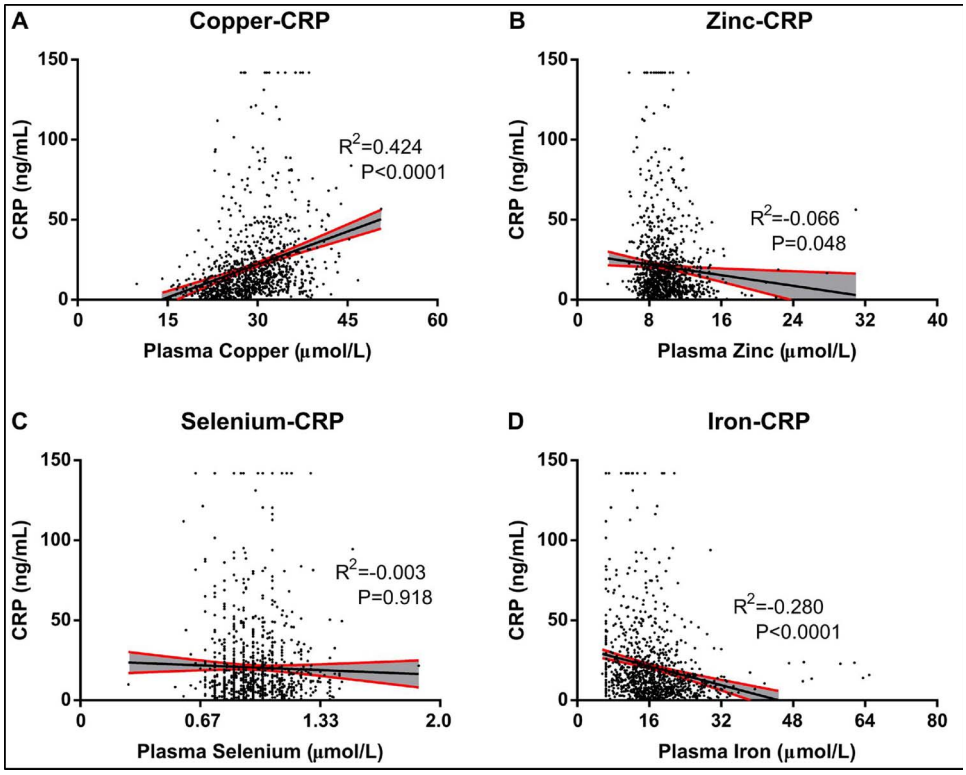

Fig. 1. Spearman's correlations between plasma trace minerals at 15 ± 1 weeks' gestation and serum CRP at 15 ± 1 weeks' gestation. A significant positive correlation was seen between plasma copper and CRP (A), whilst significant negative correlations were observed between plasma zinc (B) and plasma iron (D) with CRP. No correlation was found between plasma selenium and CRP (C).

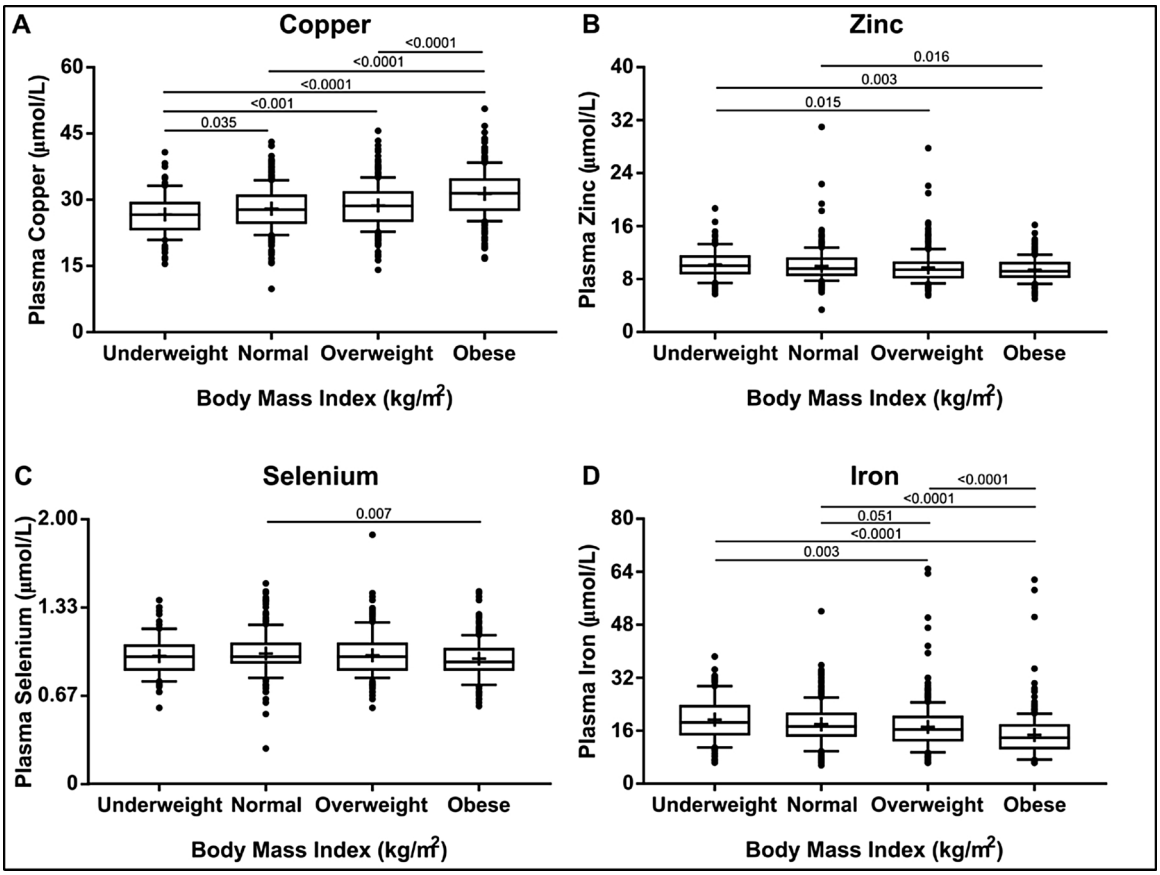

Fig. 2. Relationship between plasma trace minerals at 15 ± 1 weeks' gestation and maternal body mass index (BMI). Compared with normal weight (BMI 20.1–24.9) women, plasma copper was lower in lean (BMI ≤ 20) women, higher in obese (BMI ≥ 30) and no different in overweight (BMI 25–29.9) (A). Plasma zinc (B), plasma selenium (C) and plasma iron also did not change between normal weight and overweight women, however, were significantly lower in the obese women when compared to normal weight women. Box plots represent median: 10th–90th centile. Groups compared using a Dunn's test with a Bonferroni post-hoc test.

combined compared with women with high plasma copper (3rd tertile) (aRR = 0.77; 95% CI = 0.66, 0.91 and aRR = 0.81; 95% CI = 0.70, 0.95, respectively). When the pregnancy complications were separated, the protective effect of low plasma copper was seen in all complications except GH however, these associations were not statistically significant. Women with lower plasma zinc and selenium (1 st tertile) also had decreased risk of any pregnancy complication when compared to high plasma zinc and plasma selenium (aRR = 0.86; 95% CI = 0.74, 1.00 and aRR: 0.84; 95% CI = 0.73, 0.96, respectively). Given that the strongest effect was observed with plasma copper, we sought to assess whether an association between plasma copper status at 15 ± 1 weeks' gestation was still observed after adjust for plasma zinc and plasma selenium (Supplementary Table 1). A significant decreased risk for any complication was observed in women within the first and second tertile of plasma copper when compared to women in the third tertile indicating a relationship independent of plasma zinc or selenium status (aRR = 0.81; 95% CI = 0.69, 0.61 and aRR = 0.84; 95% CI = 0.72, 0.98, respectively). There was no significant effect of maternal iron status in early pregnancy on developing any pregnancy complication. However, women with plasma iron in the 1 st and 2nd tertile were at increased risk of GH when compared to women in the 3rd tertile (aRR = 1.78; 95% CI = 1.05, 3.02 and aRR = 1.87; 95% CI = 1.12, 3.11, respectively).

A sensitivity analysis was performed between plasma copper and CRP in order to determine the relationship between copper, inflammation and pregnancy outcome. Serum CRP measured at 15 ± 1 weeks' gestation was used as an indirect marker of inflammation. Women with CRP ≥20.3 ng/mL were excluded as normal circulating CRP ranges between 0.4–20.3 ng/mL [26] and levels higher than 20 ng/mL are often associated with infection. Supplementary Table 2 shows the effects of plasma copper status in all (n = 1065) women as well as those with CRP < 20.3 ng/mL (n = 717). The point estimates for the effects for lower plasma copper in the women whose CRP was < 20.3 ng/mL was similar to those for all women (1 st tertile: all women = 0.77 vs. women with CRP < 20.3 ng/mL = 0.74 & 2nd tertile: all women = 0.81 vs. women with CRP < 20.3 ng/mL = 0.78) indicating the possibility that the effects of plasma copper status in this study is likely to be independent of inflammation.

Given that copper and zinc share similar electro-chemical properties and biological pathways [18], the interactions between these two trace minerals was analysed in relation to pregnancy complications (Table 3). Combined low zinc status (1 st tertile) and low copper status (1 st tertile) was associated with a reduced risk of any pregnancy complication compared with high zinc status and high copper status (2nd and 3rd tertile combined for both trace minerals) (aRR = 0.72; 95% CI = 0.62, 0.85). Similarly, combined low copper and high zinc was also associated with reduced risk of any pregnancy complication when compared to combined high zinc and high copper (aRR = 0.76; 95% CI = 0.62, 0.94) suggesting copper status is a more important determinant of pregnancy outcome. When each pregnancy complication was analysed separately, a similar protective effect of combined low copper and low zinc was seen in PE, GDM, sPTB and SGA. For SGA, this was statistically significant (aRR = 0.62; 95% CI = 0.41, 0.94). For GDM, combined low copper and high zinc, as well as high copper and low zinc, also appeared to increase risk when compared to combined high copper and high zinc although these were not statistically significant (aRR = 1.46; 95% CI = 0.57, 3.69 and RR = 1.78; 95% CI = 0.77, 4.10, respectively).

Table 2
Adjusted relative risks of pregnancy complications from all complications, preeclampsia (PE), gestational hypertension (GH), gestational diabetes mellitus (GDM), spontaeous preterm birth (sPTB) and small-for-gestational age (SGA) according to plasma levels of trace minerals categorised based on population tertiles.

		Pregnancy Complication											
	All women	Any Complications		PE		GH		GDM		sPTB		SGA	
	n	*n* (%)	aRR* (95% CI)	*n* (%)	aRR* (95% CI)	*n* (%)	aRR* (95% CI)	*n* (%)	aRR* (95% CI)	*n* (%)	aRR* (95% CI)	*n* (%)	aRR* (95% CI)
Copper													
< 27.9 μmol/L	360	150 (42)	**0.77 (0.66, 0.91)**	21 (6)	0.71 (0.42, 1.18)	33 (9)	1.11 (0.71, 1.75)	13 (4)	0.69 (0.35, 1.37)	20 (6)	0.62 (0.34, 1.15)	34 (9)	**0.66 (0.44, 1.00)**
27.9 −32.5 μmol/L	349	156 (45)	**0.81 (0.70, 0.95)**	27 (8)	0.88 (0.55, 1.42)	36 (10)	1.16 (0.75, 1.80)	13 (4)	0.66 (0.33, 1.32)	16 (5)	**0.52 (0.28, 0.98)**	49 (14)	0.95 (0.66, 1.38)
> 32.5 μmol/L	356	201 (56)	1.0†	37 (10)	1.0†	39 (11)	1.0†	25 (7)	1.0†	29 (8)	1.0†	51 (14)	1.0†
Zinc													
< 8.3 μmol/L	357	160 (45)	**0.86 (0.74, 1.00)**	26 (7)	0.80 (0.48, 1.32)	42 (12)	1.01 (0.66, 1.56)	15 (4)	0.82 (0.43, 1.55)	15 (4)	0.85 (0.47, 1.52)	42 (12)	0.75 (0.52, 1.08)
8.3–9.9 μmol/L	351	165 (47)	0.91 (0.78, 1.06)	29 (8)	0.91 (0.56, 1.47)	32 (9)	0.91 (0.58, 1.43)	14 (4)	0.97 (0.50, 1.88)	27 (8)	0.91 (0.51, 1.60)	36 (10)	**0.65 (0.44, 0.96)**
> 9.9 μmol/L	356	182 (51)	1.0†	30 (8)	1.0†	34 (10)	1.0†	22 (6)	1.0†	23 (6)	1.0†	56 (16)	1.0†
Selenium													
< 0.86 μmol/L	449	203 (45)	**0.84 (0.73, 0.96)**	33 (7)	0.72 (0.45, 1.18)	49 (11)	0.94 (0.62, 1.42)	19 (4)	1.08 (0.55, 2.15)	25 (6)	0.76 (0.44, 1.31)	49 (11)	0.81 (0.55, 1.19)
0.86-0.96 μmol/L	199	83 (42)	**0.80 (0.66, 0.96)**	15 (8)	0.82 (0.45, 1.48)	16 (8)	0.77 (0.44, 1.35)	11 (6)	1.71 (0.78, 3.77)	9 (5)	0.62 (0.29, 1.33)	25 (13)	0.96 (0.61, 1.50)
> 0.96 μmol/L	417	221 (53)	1.0†	37 (9)	1.0†	43 (10)	1.0†	21 (5)	1.0†	31 (7)	1.0†	60 (14)	1.0†
Iron													
< 15.64 μmol/L	356	180 (51)	1.08 (0.92, 1.27)	31 (9)	0.81 (0.49, 1.35)	47 (13)	**1.78 (1.05, 3.02)**	19 (5)	1.16 (0.53, 2.56)	24 (7)	1.11 (0.60, 2.03)	45 (13)	1.22 (0.81, 1.83)
15.6–21.2 μmol/L	353	171 (48)	1.08 (0.92, 1.26)	26 (7)	0.87 (0.52, 1.46)	40 (11)	**1.87 (1.12, 3.11)**	20 (6)	1.46 (0.74, 2.89)	18 (5)	0.78 (0.43, 1.43)	52 (15)	1.36 (0.92, 2.01)
> 21.2 μmol/L	355	155 (44)	1.0†	28 (8)	1.0†	20 (6)	1.0†	12 (3)	1.0†	23 (6)	1.0†	37 (10)	1.0†

*Adjusted relative risks were adjusted for age, maternal body mass index and smoking status at 15 ± 1 weeks' gestation (no versus yes).
†Reference category.

3. Discussion

In this study, we have comprehensively assessed plasma trace minerals in early pregnancy and their association with multiple pregnancy complications. Contrary to previous observations of the negative impacts of poor trace element status on pregnancy outcome, we observed a protective effect of lower levels of plasma copper, zinc and selenium at the beginning of pregnancy on the risk of any pregnancy complication. However, this relationship is highly likely to be driven by copper status rather than zinc or selenium as plasma copper was found to be associated with adverse pregnancy outcome after adjusting for plasma zinc and selenium. This provides justification for further work into elucidating the role of copper in pregnancy, and the interaction with other trace elements, particularly plasma zinc, in early pregnancy.

Trace elements like copper, zinc and selenium are integral to supporting cellular and tissue physiology [27]. Indeed, animal models of copper, zinc and selenium deficiency in pregnancy consistently show negative effects on fertility, fetal growth and offspring health and wellbeing [28–32]. Copper status in pregnancy has been infrequently reported in the literature and has focused predominantly on copper deficiency as adequate supply of copper during pregnancy is necessary for early embryonic development [33]. Circulating levels of copper increase during pregnancy due to the increase in copper-carrying proteins in the blood mediated by estrogen [34,35]. Furthermore, in this

Table 3
Adjusted relative risks (aRR) of pregnancy complications from all complications, preeclampsia (PE), gestational hypertension (GH), gestational diabetes mellitus (GDM), spontaeous preterm birth (sPTB) and small-for-gestational age (SGA) based on stratification by combined plasma copper and plasma zinc concentrations.

		Pregnancy complication											
	All women	Any Complications		PE		GH		GDM		sPTB		SGA	
	n	*n* (%)	aRR* (95% CI)	*n* (%)	aRR* (95% CI)	*n* (%)	aRR* (95% CI)	*n* (%)	aRR* (95% CI)	*n* (%)	aRR* (95% CI)	*n* (%)	aRR* (95% CI)
Low Cu Low Zn	519	222 (43)	**0.72 (0.62, 0.85)**	36 (7)	0.67 (0.39, 1.16)	55 (11)	0.94 (0.57, 1.55)	17 (3)	0.71 (0.31, 1.61)	27 (5)	0.55 (0.30, 1.01)	57 (11)	**0.62 (0.41, 0.94)**
Low Cu High Zn	192	85 (44)	**0.76 (0.62, 0.94)**	13 (7)	0.75 (0.37, 1.51)	14 (7)	0.67 (0.34, 1.34)	9 (5)	1.46 (0.57, 3.69)	9 (5)	0.49 (0.21, 1.12)	27 (14)	0.81 (0.49, 1.34)
High Cu Low Zn	197	105 (53)	0.86 (0.72, 1.03)	19 (10)	0.87 (0.47, 1.61)	20 (10)	0.86 (0.47, 1.56)	17 (9)	1.78 (0.77, 4.10)	14 (7)	0.75 (0.37, 1.50)	22 (11)	0.64 (0.38, 1.07)
High Cu High Zn	156	95 (61)	1.0†	17 (11)	1.0†	19 (12)	1.0†	8 (5)	1.0†	15 (10)	1.0†	28 (18)	1.0†

Low Copper ≤32.5 μmol/L (1 st and 2nd tertile combined), High Copper > 32.5 μmol/L (3rd tertile), Low Zinc ≤9.91 μmol/L (1 st and 2nd tertile combined), High Zinc > 9.91 μmol/L (3rd tertile)
* Adjusted relative risks were adjusted for age, maternal body mass index and smoking status at 15 ± 1 weeks' gestation (no versus yes).
† Reference category.

population, circulating plasma copper was similar to pregnancy reference ranges (10–90th centile: 26.4–33.8 μmol/L) and to those reported in other populations of pregnant women [26,34,36–38]. Thus, our findings of higher copper in early pregnancy in women who subsequently develop a pregnancy complication, which are similar to other studies that have measured copper concentrations in late pregnancy [15–17], suggest higher levels of circulating copper may contribute to adverse pregnancy outcomes.

Of the three trace minerals, zinc in pregnancy has been most widely studied. In human populations, zinc deficiency in pregnancy may be associated with delivery of a low birthweight infant as well as with hypertensive disorders of pregnancy, particularly in populations of women vulnerable to inadequate zinc intakes in pregnancy [39]. However, inconsistencies between the studies on the effect of zinc deficiency in pregnancy reflects uncertainty within the literature. Furthermore, the evidence with respect to the relationship between selenium status and adverse pregnancy outcomes has not clearly been established [40]. Never-the-less, our findings that lower levels of zinc and selenium at 15 ± 1 weeks' gestation is associated with a protective effect on pregnancy outcome is in conflict with current dogma that deficiencies in these nutrients are detrimental to pregnancy health. However, given that both plasma zinc and selenium positively correlated with plasma copper, and the protective effect of lower plasma copper persisted once plasma zinc and selenium were adjusted for, implicates plasma copper status as the main contributor to adverse pregnancy outcomes in this study. This is further supported by the finding on combined copper and zinc status as whilst combined low copper and low zinc was associated with reducing the risk of all pregnancy complications, equivalently, low copper and high zinc also appeared to reduce the risk.

The greatest effect observed in this study was in the association between lower plasma copper in early pregnancy and the development of any pregnancy complication. This suggests a role for this trace mineral either within the causal pathway of, or as an early pregnancy biomarker for, adverse pregnancy outcomes. PE, GDM, sPTB and SGA are all often associated with placental dysfunction [41]. Pregnancies complicated by placental dysfunction can be characterised by impaired trophoblast invasion and transformation of the uterine spiral arteries resulting in inadequate uteroplacental blood flow or compromised fetal placental vasculature development [42]. It is still unclear as to what causes the initial failure of the placenta to form correctly. However, it is likely to be a combination of both genetic and environmental factors with increased oxidative and cellular stress, as well as inflammatory mediators, thought to be key [43]. Elevated free copper can be a source of oxidative stress [44] and thus, it is possible that elevated copper may be a potential contributor to increased oxidative stress in the placenta, particularly in early pregnancy.

Another potential hypothesis is that early pregnancy copper status may be a biomarker for an increased inflammatory response which itself predisposes women to developing a pregnancy complication. Pregnancy itself is considered a pro-inflammatory state, particularly during the peri-implantation period [45]. However, even in pregnancy, the inflammation response is tightly regulated and slight deviations can result in compromised tissue function. Indeed, inflammation, as well as obesity which induces persistent low-grade inflammation, are associated with increasing the risk of a number of pregnancy complications like PE and GDM [46–49]. However, we observed a protective effect of lower copper concentrations on the risk of pregnancy complications after adjusting for BMI. Furthermore, in the sensitivity analysis, in which all women with a possible infection or exacerbated inflammatory response were excluded, very little change to the point estimates for copper was found when compared to estimates calculated in the whole cohort suggesting plasma copper is not necessarily just a biomarker for inflammation.

The overall aim of this study was to determine whether early pregnancy plasma trace mineral status was associated with pregnancy complications. Despite the tight biological regulation of these minerals in the circulation [50], lower levels of plasma copper, zinc and selenium may be protective against a number of the major pregnancy complications assessed. However, there are a number of limitations, most notably lack of high quality dietary intake data, which may have implications for the results of this study. Plasma and serum measures are not generally considered accurate determinants of nutrient status [51] and non-fasting blood samples were collected which is also likely to have an effect on short-term mineral status. Although, it is possible that higher circulating levels of these trace elements represent reduced bioavailability within cells, where the minerals are required. Hence, it is conceivable that lower circulating levels of copper, zinc and selenium may be associated with a reduced risk of pregnancy complications as more of the trace elements are being utilised with tissues where required. Furthermore, given the lack of suitable alternatives in which to determine women's nutritional status in this pregnancy cohort, measuring plasma minerals may still be informative in understanding the physiology of pregnancy and pregnancy complications.

In conclusion, we observed a very clear association between plasma copper concentrations in early pregnancy and the risk of pregnancy complications that warrants further investigation particularly within the context of other trace element status. It is conceivable that elevated plasma copper status at the beginning of pregnancy may simply be a biomarker of inflammation which may underlie the development of a particular pregnancy complication. However, our data suggest it is more likely to have a mechanistic role, potentially in the placenta through oxidative stress or inflammatory pathways that warrants further investigation. Much could be gained if we expand our understanding of maternal nutrition in pregnancy to incorporate simultaneous mechanistic and epidemiological studies, particularly on multiple micronutrients and their interactions in order to fully elucidate the importance of these factors in pregnancy success.

Conflicts of interest

The authors declare no conflicts of interest relating to this manuscript.

Funding

CTR is supported by a Lloyd Cox Obstetrics and Gynaecology Professorial Research Fellowship, University of Adelaide. LEG is supported by an Australian National Health and Medical Centre (NHMRC) Early Career Fellowship (APP1070421). RLW is supported by an Australian Postgraduate Award. Trace element analyses were funded by a Lloyd Cox Research Excellence Award University of Adelaide to CTR.

Acknowledgments

We would like to thank John Gouzos and Claire Wright at the Analytical Services Unit (ASU) in CSIRO Land and Water, Waite Campus, University of Adelaide for their time, assistance and expertise in measuring the plasma minerals.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jtemb.2017.11.016.

References

[1] C.A. Maloney, W.D. Rees, Gene-nutrient interactions during fetal development, Reproduction 130 (4) (2005) 401–410.
[2] R.E. Black, Micronutrients in pregnancy, Br. J. Nutr. 85 (Suppl 2) (2001) S193–197.
[3] C.T. Roberts, IFPA Award in Placentology Lecture: complicated interactions between genes and the environment in placentation, pregnancy outcome and long term health, Placenta 31 (Suppl) (2010) S47–53.

[4] P. Christian, C.P. Stewart, Maternal micronutrient deficiency, fetal development, and the risk of chronic disease, J. Nutr. 140 (3) (2010) 437–445.
[5] A.D. Gernand, K.J. Schulze, C.P. Stewart, K.P. West Jr., P. Christian, Micronutrient deficiencies in pregnancy worldwide: health effects and prevention, Nat. Rev. Endocrinol. 12 (5) (2016) 274–289.
[6] L.S. Hurley, Teratogenic aspects of manganese, zinc, and copper nutrition, Physiol. Rev. 61 (2) (1981) 249–295.
[7] S. Cunningham-Rundles, D.F. McNeeley, A. Moon, Mechanisms of nutrient modulation of the immune response, J. Allergy Clin. Immunol. 115 (6) (2005) 1119–1128 (quiz 1129).
[8] C.W. Redman, G.P. Sacks, I.L. Sargent, Preeclampsia: an excessive maternal inflammatory response to pregnancy, Am. J. Obstet. Gynecol. 180 (2 Pt 1) (1999) 499–506.
[9] G.J. Burton, H.W. Yung, T. Cindrova-Davies, D.S. Charnock-Jones, Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia, Placenta 30 (Suppl A) (2009) S43–48.
[10] P.J. Shen, B. Gong, F.Y. Xu, Y. Luo, Four trace elements in pregnant women and their relationships with adverse pregnancy outcomes, Eur. Rev. Med. Pharmacol. Sci. 19 (24) (2015) 4690–4697.
[11] G. Zheng, H. Zhong, Z. Guo, Z. Wu, H. Zhang, C. Wang, Y. Zhou, Z. Zuo, Levels of heavy metals and trace elements in umbilical cord blood and the risk of adverse pregnancy outcomes: a population-based study, Biol. Trace Elem. Res. 160 (3) (2014) 437–444.
[12] V.B. Rezende, F. Barbosa Jr., A.C. Palei, R.C. Cavalli, J.E. Tanus-Santos, V.C. Sandrim, Correlations among antiangiogenic factors and trace elements in hypertensive disorders of pregnancy, J. Trace Elem. Med. Biol. 29 (2015) 130–135.
[13] N. Al-Jameil, H. Tabassum, H. Al-Mayouf, H.I. Aljohar, N.D. Alenzi, S.M. Hijazy, F.A. Khan, Analysis of serum trace elements-copper, manganese and zinc in preeclamptic pregnant women by inductively coupled plasma optical emission spectrometry: a prospective case controlled study in Riyadh, Saudi Arabia, Ind. J. Clin Exp. Pathol. 7 (5) (2014) 1900–1910.
[14] V. Fenzl, Z. Flegar-Mestric, S. Perkov, L. Andrisic, F. Tatzber, N. Zarkovic, Z. Duic, Trace elements and oxidative stress in hypertensive disorders of pregnancy, Arch. Gynecol. Obstet. 287 (1) (2013) 19–24.
[15] L. Bermudez, C. Garcia-Vicent, J. Lopez, M.I. Torro, E. Lurbe, Assessment of ten trace elements in umbilical cord blood and maternal blood: association with birth weight, J. Transl. Med. 13 (2015) 291.
[16] R. Choi, J. Sun, H. Yoo, S. Kim, Y.Y. Cho, H.J. Kim, S.W. Kim, J.H. Chung, S.Y. Oh, S.Y. Lee, A prospective study of serum trace elements in healthy Korean pregnant women, Nutrients 8 (11) (2016).
[17] Y. Atamer, Y. Kocyigit, B. Yokus, A. Atamer, A.C. Erden, Lipid peroxidation, antioxidant defense, status of trace metals and leptin levels in preeclampsia, Eur. J. Obstet. Gynecol. Reprod. Biol. 119 (1) (2005) 60–66.
[18] I. Bremner, J.H. Beattie, Copper and zinc metabolism in health and disease: speciation and interactions, Proc. Nutr. Soc. 54 (2) (1995) 489–499.
[19] L.M. McCowan, C.T. Roberts, G.A. Dekker, R.S. Taylor, E.H. Chan, L.C. Kenny, P.N. Baker, R. Moss-Morris, L.C. Chappell, R.A. North, Risk factors for small-for-gestational-age infants by customised birthweight centiles: data from an international prospective cohort study, BJOG 117 (13) (2010) 1599–1607.
[20] L.C. Kenny, M.A. Black, L. Poston, R. Taylor, J.E. Myers, P.N. Baker, L.M. McCowan, N.A. Simpson, G.A. Dekker, C.T. Roberts, K. Rodems, B. Noland, M. Raymundo, J.J. Walker, R.A. North, Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study, Hypertension 64 (3) (2014) 644–652.
[21] L.M. McCowan, G.A. Dekker, E. Chan, A. Stewart, L.C. Chappell, M. Hunter, R. Moss-Morris, R.A. North, Spontaneous preterm birth and small for gestational age infants in women who stop smoking early in pregnancy: prospective cohort study, BMJ 338 (2009) b1081.
[22] A.L. Tranquilli, G. Dekker, L. Magee, J. Roberts, B.M. Sibai, W. Steyn, G.G. Zeeman, M.A. Brown, The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP, Pregnancy Hypertens 4 (2) (2014) 97–104.
[23] International Association of D, Pregnancy Study Groups Consensus P, B.E. Metzger, S.G. Gabbe, B. Persson, T.A. Buchanan, P.A. Catalano, P. Damm, A.R. Dyer, A. Leiva, M. Hod, J.L. Kitzmiler, L.P. Lowe, H.D. McIntyre, J.J. Oats, Y. Omori, M.I. Schmidt, International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy, Diabetes Care 33 (3) (2010) 676–682.
[24] R Development Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2011.
[25] P. Davis, K. McLeod, M. Ransom, P. Ongley, N. Pearce, P. Howden-Chapman, The New Zealand Socioeconomic Index: developing and validating an occupationally-derived indicator of socio-economic status, Aust. N. Z. J. Public Health 23 (1) (1999) 27–33.
[26] M. Abbassi-Ghanavati, L.G. Greer, F.G. Cunningham, Pregnancy and laboratory studies: a reference table for clinicians, Obstet. Gynecol. 114 (6) (2009) 1326–1331.
[27] F.H. Nielsen, New essential trace elements for the life sciences, Biol. Trace Elem. Res. 2 (1990) 6–27 (599–611).
[28] L.S. Hurley, P.B. Mutch, Prenatal and postnatal development after transitory gestational zinc deficiency in rats, J. Nutr. 103 (5) (1973) 649–656.
[29] J.Y. Uriu-Adams, C.L. Keen, Zinc and reproduction: effects of zinc deficiency on prenatal and early postnatal development, Birth Defects Res. B Dev. Reprod. Toxicol. 89 (4) (2010) 313–325.
[30] M.E. Beckers-Trapp, L. Lanoue, C.L. Keen, R.B. Rucker, J.Y. Uriu-Adams, Abnormal development and increased 3-nitrotyrosine in copper-deficient mouse embryos, Free Radic. Biol. Med. 40 (1) (2006) 35–44.
[31] F. Nogales, M.L. Ojeda, M. Fenutria, M.L. Murillo, O. Carreras, Role of selenium and glutathione peroxidase on development, growth, and oxidative balance in rat offspring, Reproduction 146 (6) (2013) 659–667.
[32] A.R. Menino Jr., W.S. Damron, T.E. Henry, J.L. O'Claray, The influence of dietary copper on reproduction, growth and the cardiovascular system in Swiss-Webster female mice, Lab. Anim. Sci. 36 (2) (1986) 164–167.
[33] C.L. Keen, J.Y. Uriu-Hare, S.N. Hawk, M.A. Jankowski, G.P. Daston, C.L. Kwik-Uribe, R.B. Rucker, Effect of copper deficiency on prenatal development and pregnancy outcome, Am. J. Clin. Nutr. 67 (5 Suppl) (1998) 1003S–1011S.
[34] S. Izquierdo Alvarez, S.G. Castanon, M.L. Ruata, E.F. Aragues, P.B. Terraz, Y.G. Irazabal, E.G. Gonzalez, B.G. Rodriguez, Updating of normal levels of copper, zinc and selenium in serum of pregnant women, J. Trace Elem. Med. Biol. 21 (Suppl 1) (2007) 49–52.
[35] J. Raymunt, E.M. Russ, Influence of estrogens on total serum copper and caeruloplasmin, Proc. Soc. Exp. Biol. Med. 92 (3) (1956) 465–466.
[36] H.D. Mistry, L.O. Kurlak, S.D. Young, A.L. Briley, F.B. Pipkin, P.N. Baker, L. Poston, Maternal selenium, copper and zinc concentrations in pregnancy associated with small-for-gestational-age infants, Matern. Child Nutr. 10 (3) (2014) 327–334.
[37] E. Al-Saleh, M. Nandakumaran, M. Al-Shammari, F. Al-Falah, A. Al-Harouny, Assessment of maternal-fetal status of some essential trace elements in pregnant women in late gestation: relationship with birth weight and placental weight, J. Matern. Fetal Neonatal. Med. 16 (1) (2004) 9–14.
[38] P. Pathak, U. Kapil, S.K. Kapoor, R. Saxena, A. Kumar, N. Gupta, S.N. Dwivedi, R. Singh, P. Singh, Prevalence of multiple micronutrient deficiencies amongst pregnant women in a rural area of Haryana, Indian J. Pediatr. 71 (11) (2004) 1007–1014.
[39] R.L. Wilson, J.A. Grieger, T. Bianco-Miotto, C.T. Roberts, Association between maternal zinc status, dietary zinc intake and pregnancy complications: a systematic review, Nutrients 8 (10) (2016).
[40] M.P. Rayman, D. Hatfield, U. Schweizer, P. Tsuji, V. Gladyshev (Eds.), Is Adequate Selenium Important for Healthy Human Pregnancy? Springer, Cham, Selenium, 2016.
[41] I. Brosens, R. Pijnenborg, L. Vercruysse, R. Romero, The great Obstetrical Syndromes are associated with disorders of deep placentation, Am. J. Obstet. Gynecol. 204 (3) (2011) 193–201.
[42] Y. Khong, I. Brosens, Defective deep placentation, Best Pract. Res. Clin. Obstet. Gynaecol. 25 (3) (2011) 301–311.
[43] E. Jauniaux, L. Poston, G.J. Burton, Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution, Hum. Reprod. Update 12 (6) (2006) 747–755.
[44] N. Shukla, C.S. Thompson, G.D. Angelini, D.P. Mikhailidis, J.Y. Jeremy, Low micromolar concentrations of copper augment the impairment of endothelium-dependent relaxation of aortae from diabetic rabbits, Metabolism 53 (10) (2004) 1315–1321.
[45] G. Mor, I. Cardenas, V. Abrahams, S. Guller, Inflammation and pregnancy: the role of the immune system at the implantation site, Ann. N. Y. Acad. Sci. 1221 (2011) 80–87.
[46] S.Y. Chu, W.M. Callaghan, S.Y. Kim, C.H. Schmid, J. Lau, L.J. England, P.M. Dietz, Maternal obesity and risk of gestational diabetes mellitus, Diabetes Care 30 (8) (2007) 2070–2076.
[47] C. Qiu, T.K. Sorensen, D.A. Luthy, M.A. Williams, A prospective study of maternal serum C-reactive protein (CRP) concentrations and risk of gestational diabetes mellitus, Paediatr. Perinat. Epidemiol. 18 (5) (2004) 377–384.
[48] L.M. Bodnar, R.B. Ness, N. Markovic, J.M. Roberts, The risk of preeclampsia rises with increasing prepregnancy body mass index, Ann. Epidemiol. 15 (7) (2005) 475–482.
[49] L.M. Bodnar, R.B. Ness, G.F. Harger, J.M. Roberts, Inflammation and triglycerides partially mediate the effect of prepregnancy body mass index on the risk of preeclampsia, Am. J. Epidemiol. 162 (12) (2005) 1198–1206.
[50] J.F. Collins, Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals, (2016).
[51] M. Hambidge, Biomarkers of trace mineral intake and status, J. Nutr. 133 (Suppl 3) (2003) 948S–955S.