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Maternal selenium, copper and zinc concentrations in pregnancy associated with small-for-gestational-age infants.

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Running Title: Micronutrient concentrations, SGA and adolescence.

Abstract

Pregnancy during adolescence increases the risk of adverse pregnancy outcome, especially risk of small-for gestational-age (SGA) birth, which has been linked to micronutrient deficiencies. Likewise, smoking has been shown to be related with lower micronutrient concentrations. Different ethnicities have not previously been examined. We used a subset from a prospective observational study, the About Teenage Eating (ATE) study consisting of 126 pregnant adolescents (14-18 years old) between 28-32 weeks' gestation. Micronutrient status was assessed by inductively-coupled mass spectrometry. Smoking was assessed by self-report and plasma cotinine, and SGA was defined as infants born < 10th corrected birthweight centile. The main outcome measures were: 1) Maternal plasma selenium, copper and zinc concentrations in adolescent mothers giving birth to SGA *versus* appropriate-for-gestational-age (AGA) infants. 2) Comparison of micronutrient concentrations between women of different ethnicities and smoking habits. The plasma selenium (mean ± SD [95% CI]) concentration was lower in the SGA (n = 19: 49.4 ± 7.3 [CI: 45.9, 52.9] μg/L) compared to the AGA (n = 107: 65.1 ± 12.5 [CI: 62.7, 67.5] μg/L; $P < 0.0001$) group. Smoking mothers had a lower selenium concentration compared to non-smokers ($P = 0.01$) and Afro-Caribbean women had higher selenium concentrations compared to White Europeans ($P = 0.02$). Neither copper nor zinc concentrations varied between groups, but selenium and copper were moderately correlated ($P < 0.05$). Selenium is an essential trace element which exerts its biological effects through the expression of a variety of important selenoproteins. Low plasma selenium concentration in adolescent mothers could contribute to the risk of delivering an SGA infant, possibly through lowering the placental antioxidant defence, thus direclty affecting fetal growth. The differences in plasma selenium between different ethnicities may relate to variation in nutritional intake, which requires further investigation.

Keywords: Micronutrients, small-for-gestational-age, adolescence

Introduction

Worldwide, pregnancies during adolescence are associated with a high risk of an adverse obstetric outcome, particularly small-for-gestational-age (SGA) birth delivery (Chen et al., 2007). Although teenage pregnancy rates in the United Kingdom have fallen by 3.1% since 2007, they remain amongst the highest in Western Europe (40.6 births per 1,000 women aged 15-17 in 2008 in England and Wales) (Office of National Statistics, 2010).

Pregnant adolescents in industrialised countries typically have a poor diet, may attributable to their age and socio-economic background (Moran, 2007) and nutrient intake in this population has been shown to be inadequate (Crawley, 1993). A recent study by our group (the About Teenage Eating (ATE) study) carried out in two inner city populations in the United Kingdom reported a high rate of SGA infants in teenage pregnancies and demonstrated a strong association with reduced folate status (Baker et al., 2009). In this study, we have investigated the status of 3 essential antioxidant micronutrients previously associated with poor pregnancy outcome: selenium, copper and zinc (Mistry and Williams, 2011).

Selenium, an essential trace element, is a co-factor for several important enzymes that play a focal role in antioxidant defence including the glutathione peroxidases (GPxs), which metabolise the products of attack by hydrogen peroxidases and oxidised lipoproteins (Rayman, 2000). Selenium also has both structural and enzymatic roles and functions as a catalyst for the production of thyroid hormones (Beckett and Arthur, 2005). In a recent study we reported that selenium concentrations were low in women of reproductive age in the United Kingdom, falling further during pregnancy and this correlated with low plasma and placental GPx activities (Mistry et al., 2008). Selenium deficiency has also been linked with several reproductive complications, including SGA infants (Mistry et al., 2012, Mariath

et al., 2011, Klapec et al., 2008, Strambi et al., 2004). It has also been reported that blood selenium concentrations are lower in tobacco smokers (Northrop-Clewes and Thurnham, 2007).

Copper is an essential cofactor for a number of enzymes involved in metabolic reactions, angiogenesis, oxygen transport and antioxidant protection, including catalase, and copper/zinc superoxide dismutase (Cu/Zn SOD) (Gambling et al., 2008). During pregnancy, plasma copper concentrations significantly increase, returning to normal non-pregnant values after delivery (Izquierdo Alvarez et al., 2007). This increase could be partly related to synthesis of ceruloplasmin, a major copper-binding protein, due to altered levels of oestrogen (Izquierdo Alvarez et al., 2007). Lower copper concentrations have been reported in placentae of SGA pregnancies (Zadrozna et al., 2009), but there is limited data regarding maternal plasma copper concentrations in relation to SGA pregnancies.

Zinc is an essential constituent of over 200 metalloenzymes, and participates in carbohydrate and protein metabolism, nucleic acid synthesis, and antioxidant functions (through Cu/Zn SOD) (Izquierdo Alvarez et al., 2007). It has been estimated that the total amount of zinc retained during pregnancy is ~ 100 mg (Swanson and King, 1987). The requirement for zinc during the third trimester is approximately twice as high as that in non-pregnant women (WHO/FAO/IAEA, 1996). Plasma zinc concentrations decline as pregnancy progresses and then paradoxically increase towards delivery (Izquierdo Alvarez et al., 2007). Zinc supplementation during pregnancy has been reported to significantly increase birthweight and head circumference (Goldenberg et al., 1995), highlighting the importance of adequate zinc supply during pregnancy.

Failure to achieve genetic growth potential is a major cause of perinatal morbidity and mortality and is estimated to occur in 10% of pregnancies in the developed world and up to 25% in undeveloped countries (Steer, 2005). These complications are increasingly evident at lower birthweight centiles. The mechanisms are still to be elucidated but a likely common aetiological factor for SGA is placental ischemia/hypoxia (Biri et al., 2007), which would be associated with oxidative stress. There are few studies of selenium concentrations in SGA/fetal growth restricted births (Klapec et al., 2008, Llanos and Ronco, 2009) and none specifically addressing adolescent pregnancies; there is a similar lack of information about copper and zinc. A reduced micronutrient concentration may lead to inadequate antioxidant protection culminating in poor fetal growth. Ischemia-reperfusion injury may contribute to the oxidative stress and could result in the release of reactive oxygen species into the maternal circulation possibly resulting in oxidative DNA damage which may underlie development of SGA (Takagi et al., 2004).

We hypothesised that the micronutrient concentrations would be reduced in mothers who delivered SGA infants. Due to potential differences in nutritional intakes, we further hypothesised that differences in micronutrient concentrations would be observed between White European and Afro-Caribbean adolescent pregnant women. Since it is well-documented that smoking has a detrimental effect on fetal growth (Kho et al., 2009), associations with micronutrient concentrations and smoking habits in the pregnant adolescents were also explored.

The aim of this study, therefore, was to establish the maternal plasma selenium, zinc and copper in adolescent mothers delivering SGA and AGA infants and use these data to investigate any differences in these antioxidant micronutrients between ethnicities and smoking status.

Methods

Subjects: The 126 women contributing to the present study represent a sub-group of the larger ATE study of 500 adolescents from whom samples of adequate volume were available (Baker et al., 2009). The study was approved by the Central Manchester Local Research Ethics Committee (local registration no. 03/CM/032) and informed written consent was obtained from all participants; pregnant adolescents 14-18 years old singleton pregnancies were assessed for capacity to provide informed consent according to accepted United Kingdom criteria (Gillick v West Norfolk & Wisbech, 1985). In order to minimise potential confounding effects of different socioeconomic background and lifestyle between Manchester and London, we studied only those 126 pregnant adolescent women recruited to the ATE study between 2004 and 2007 at 2 hospitals in South London, United Kingdom. Exclusion criteria were: inability to provide informed consent, pre-eclampsia in previous pregnancy, clotting disorders, HIV/AIDS, haemoglobinopathies, known pre-existing diabetes, renal disease, hypertension, multiple pregnancies, or a history of ≥ 1 previous miscarriage. SGA was defined as individualised birthweight ratio below the 10^{th} percentile (American College of Obstetricians and Gynecologists, 2000) and calculated using the customised birthweight centiles (Gardosi and Francis, 2006). In addition, birthweight *z* scores were calculated corrected for gestational age at delivery from the UK WHO 2006 growth charts (Cole et al., 2011).

Sample collection and laboratory methods: A 30 ml, non-fasting sample of venous blood was collected in the early third trimester (mean ± SD: 30.3 ± 2.1 weeks' gestation) into chilled collection tubes. Blood samples were transported on ice to the laboratory and centrifuged at 4^{o}C within 30 minutes of collection. Plasma was stored at -80^{o}C until analysis.

Plasma concentrations of copper, zinc and selenium in plasma were assayed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at m/z 65, 66 and 78 respectively. Samples and standards (SPEX Certiprep Inc.) were prepared identically in a diluent containing 0.1% 'Triton X-100' non-ionic surfactant (+'antifoam-B', Sigma), 2% methanol and 1% HNO_3 (trace analysis grade) including the internal ICP-MS standards Iridium (5 µg L^{-1}), Rhodium (10 µg L^{-1}), Gallium (25 µg L^{-1}) and Scandium (50 µg L^{-1}). For all three analytes, the ICP-MS was run in 'collision-reaction cell mode' with pure H_2 as the cell gas to maximise sensititvity for ^{78}Se determination. Aspiration was through a single sample line via a Burgener-Miramist PEEK nebuliser. Calibrations for all micronutrients were in the range 0 – 50 µg L^{-1}. Quality of analysis was assured by the use of appropriate reference materials (Seronorm and UTAK; Nycomed Pharma AS). Trace element free techniques were used during collection and analysis, following guidelines from the International Zinc Nutrition consultative group (IZiNCG). Both intra- and inter-assay coefficients of variances were < 5%.

Smoking history was ascertained by direct questioning and verified by plasma cotinine, measured by solid-phase competitive chemiluminescence immunoassay (DPC, Gwynedd, UK). Responses were coded as smokers or non-smokers, which included ex-smokers.

Statistical analysis: All tests were performed using SPSS for Windows version 16.0. Data were tested for normality of distribution using the Kolmogorov-Smirnov test. Summary data are presented as mean ± SD depending. Between-group comparisons were made using Student's *t* tests. Multiple logistic regression models for AGA/SGA with selenium, smoking and ethnicity individually and together were also conducted. Pearson's correlation test was used to test associations. The null hypothesis was rejected where $P < 0.05$.

Results

Subjects: Table 1 describes the demographic, obstetric and pregnancy outcome data of the 126 women for whom blood samples were available. More detailed descriptions have been previously published (Baker et al., 2009). The two ethnic groups were well-matched for age and BMI; the sub group showed no significant difference in any outcome variable when compared to the remaining study population (Baker et al., 2009). By definition, both the bithweights and customised birthweight centiles were significantly lower in the SGA group (Table 1).

SGA: Nineteen mothers delivered SGA infants and the median corrected birthweight centiles for all infants in this study were below the 50^{th} centile (Table 1). The plasma selenium concentration (mean ± SD [95% CI]) was lower in the mothers who gave birth to SGA infants (49.4 ± 7.3 [CI: 45.9, 52.9] μg/L) compared to the AGA infants (65.1 ± 12.5 [CI: 62.7, 67.5] μg/L; $P < 0.0001$; Figure 1). Furthermore, a significant positive association was observed between selenium concentrations and birthweight *z* scores (r = 0.203; $P = 0.03$; Figure. 2). No differences were observed between groups for copper or zinc ($P > 0.05$ for both).

Smoking: Serum selenium showed smokers (verified by plasma cotinine) had lower plasma selenium concentrations (n = 89) compared to non-smokers (n = 37; $P = 0.01$; Table 2). No significant differences were observed for copper or zinc ($P > 0.05$).

Ethnicity: Plasma micronutrient concentrations were compared between White European (n = 66) and Afro-Caribbean (n = 60) mothers. The selenium concentration was lower in White-European compared to Afro-Caribbean women ($P = 0.02$; Table 2). No differences were found in the copper or zinc concentration ($P > 0.05$; Table 2).

Multiple logistic regression models indicated selenium as a strong influencing factor and the addition of ethnicity strengthened this; however smoking and ethnicity individually had no effect (Table 3).

Discussion

This study reports lower plasma selenium concentration, but not copper or zinc in adolescent mothers delivering SGA infants. Selenium deficiency has been associated with obstetric complications including pre-eclampsia (Mistry et al., 2008), preterm birth (Dobrzynski et al., 1998) and delivery of SGA infants (Klapec et al., 2008). Small size at birth has been postulated to increase the risks of cardiovascular disease in later life and these obstetric complications are increased in adolescent pregnancies (Chen et al., 2007), further highlighting the need to investigate this important population.

This study is the first to present data linking reduced maternal plasma selenium, with SGA births in adolescent pregnancies from the United Kingdom. We have previously shown that selenium concentrations fall during pregnancy indicating an increased requirement for selenium in pregnancy as a result of the demands from the growing fetus (Mistry et al., 2008) and possibly altered intestinal re-absorption or renal handling (Szybinski et al., 2010). This reduced selenium concentration might adversely affect the functional activities of the antioxidant selenoproteins as we have shown previously (Mistry et al., 2010), compromising protection against placental oxidative stress, thus detrimentally impacting on fetal growth, although placental selenium concentrations are not known. The calculated plasma selenium concentration required for maximal plasma GPx activity in non-pregnant adult humans has been estimated to be ~90 μg/L (Duffield et al., 1999), considerably higher than the concentrations observed in the teenage mothers of this study, especially those delivering SGA infants. One factor that could contribute to the lower selenium concentration is the

decline in selenium content of flour in the United Kingdom, since the European Union reduced imports of wheat from the USA and Canada, where selenium content of the soil is higher (Jackson et al., 2004). A limitation of this study is that baseline, pre-pregnancy selenium concentrations were not available, thus we were not able to ascertain if the adolescents that went on to deliver an SGA infant started with lower selenium concentrations compared to those delivering AGA infants.

Recent reports from Europe and the USA have suggested that blood selenium concentrations are lowered in tobacco smokers (Northrop-Clewes and Thurnham, 2007, Galan et al., 2005). Smoking is associated with decreased food intake, which could itself result in decreased selenium status. Furthermore, tobacco smoking causes inflammation and induces oxidative stress and the lower selenium concentration may contribute to these factors (Galan et al., 2005, Northrop-Clewes and Thurnham, 2007, Ellingsen et al., 2009). Another possibility is that the increased exposure of smokers to the heavy metal cadmium might decrease the bioavailability of selenium (Galan et al., 2005, Northrop-Clewes and Thurnham, 2007).

In this adolescent pregnant cohort, a combination of poor eating habits and tobacco smoking may have amplified any reduction in the plasma selenium concentration (Baker et al., 2009). This is further substantiated by the finding of Galan *et al* that women of younger age had a low mean selenium concentration, which were further influenced by nutrient intakes and smoking (Galan et al., 2005). We anticipated that because of the characteristic poor diet in this population (Baker et al., 2009), the copper, selenium and zinc concentrations would be lower than older mothers, however our data does not support this as similar levels were found to that previously reported in slightly older White European primigravidae (Mistry et al., 2008); this may reflect the general decline in selenium intake in this population from the

United Kingdom. Future prospective studies of age related profile of selenium concentrations including adolescents and older mothers would be of interest in this regard. The differences in selenium concentrations between different ethnicities may be related to the nutritional intakes in women from different cultural backgrounds (Kant and Graubard, 2007). Studies of selenium concentrations relating to ethnic differences in a United Kingdom cohort have yet to be completed.

A limitation of this study is a large proportion of the women for whom samples were available, were 17-18 years in age; future follow-up work is required focussing on the more vulnerable younger adolescents (12-16 years). Also, the numbers in this study were small and thus future studies with larger sample sizes and a wider spread of ethnicities and measurements of the respective micronutrient antioxidant activities (GPxs and SODs) are required to confirm these initial results. The results of our study highlight the importance of monitoring maternal nutrition, particularly the micronutrient selenium intake and concentrations during adolescent pregnancies. Prenatal guidance needs to be made clear to ensure that women and practioners are aware of the nutritional requirements during pregnancy, and how healthy diet can prevent diseases of pregnancy in this venerable high risk adolescent group. This study provides preliminary evidence on the importance of proper education of good nutrition and the potential need for future selenium supplementation studies.

Key Message

1) Maternal selenium concentrations are significantly lower in adolescent pregnant women delivering SGA infants compared to those delivering AGA infants.
2) Further research is needed to accurately quantify levels of micronutrients in adolescent pregnancies and how levels vary over the course of pregnancy.

3) The actions of antioxidant micronutrient activities on maternal, fetal and placental health during adolescence need to further elucidated.

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Contributions: The authors' contributions were as follows: HDM formulated and organised the project, analysed the data and wrote the majority of the manuscript; FBP assisted in the data analysis; SDY and LOK coordinated/ ran the selenium assays; ALB assisted with sample identification and transporting samples; LP and PNB were principal investigators and designed the ATE study.

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401 **Table 1.** Demographic, obstetric and pregnancy data of subject groups used in the study.
402 Data represented as means ± SD or median [IQR] as appropriate, except for preterm births,
403 smoking status, parity, ethnicity, and Caesarean sections which are shown as number
404 (percentage).

Parameter	**AGA** **n = 107**	**SGA** **n = 19**
Age (yrs) (Mean ± SD)	17.5 ± 0.7	17.6 ± 0.8
Ethnic group [n (%)]		
White European	61 (57)	5 (26)
Afro-Caribbean	46 (43)	14 (74)
Booking body mass index (Kg/m^2) (Mean ± SD)	24.2 ± 5.2	25.6 ± 5.5
Smoking status [n (%)]		
Non-smoker	77 (72)	12 (63)
Smoker	30 (28)	7 (37)
Parity [n (%)]		
Nulliparous	102 (95)	19 (100)
Multiparous	5 (5)	0 (0)
Gestational age at delivery (Wks) (Mean ± SD)	39.8 ± 1.7	38.9 ± 2.7
Mean birthweight (g) (Mean ± SD)	3344 ± 521	2399 ± 456
Corrected birthweight centile (median [IQR])	47.1 [27, 68.2]	0.2 [0.6, 8.4]
Preterm [n (%)]	10 (9)	2 (11)
Caesarean Section [n (%)]	19 (18)	5 (26)

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406 **Table 2.** The plasma selenium, copper and zinc concentration (mean ± SD [95% CI]) in
407 adolescent mothers delivering spilt by ethnicity and smoking habit; * $P < 0.05$ between
408 ethnicity and smoking habit for selenium only.

	Selenium (µg/L)	Copper (µg/L)	Zinc (µg/L)
White European	60.3 ± 9.5 [CI: 58.0, 62.7]	2021.7 ± 365.2 [CI: 1931.9, 2111.4]	646.8 ± 230.9 [CI: 590.0, 703.5]
Afro-Caribbean	65.9 ± 16.3 * [CI: 61.7, 70.1]	2068.3 ± 402.4 [CI: 1965.2, 2171.4]	642.3 ± 365.6 [CI: 548.7, 735.9]
Non-Smoker	64.6 ± 13.2 [CI: 61.9, 67.4]	2029.8 ± 366.5 [CI: 1952.6, 2107.0]	647.5 ± 340.2 [CI: 575.9, 719.2]
Smoker	58.1 ± 11.8 * [CI: 54.1, 62.0]	2080.3 ± 426.9 [CI: 1937.9, 2222.6]	640.5 ± 189.4 [CI: 577.5, 703.6]

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Table 3: Multiple logistic regression analysis of AGA/SGA with covariate selenium and factors ethnicity and smoking.

Covariate	**Factor(s)**	χ^2	***P***
Selenium		29.5	<0.0001
Selenium	Ethnicity & Smoking	38.1	<0.0001
Selenium	Ethnicity	37.2	<0.0001
	Ethnicity	3.3	0.07
	Smoking	0.6	0.435

Figure 1. The maternal plasma selenium concentration (mean ± SD) in mothers giving birth to SGA or AGA infants; ***P <0.0001 between groups.

Figure 2. Scatter plot demonstrating the association between maternal plasma selenium concentration and birthweight z scores (r = 0.203; R^2 = 0.041; P = 0.03).