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Early childhood wheezing symptoms in relation to plasma selenium in pregnant mothers and neonates

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Summary

Objective Reduced dietary selenium intake has been linked to the development of asthma. We have investigated whether childhood wheezing symptoms, and asthma up to the age of 5 years are associated with plasma selenium and erythrocyte glutathione peroxidase (GPx) concentrations in pregnant mothers and neonates.

Methods Two thousand pregnant women were recruited and their 1924 singleton children followed up. Plasma selenium and erythrocyte GPx concentrations were measured in maternal blood during early pregnancy (12 weeks gestation) and in neonatal cord blood. Cohort children were followed up at 1, 2 and 5 years using a respiratory symptom questionnaire and at 5 years children were also invited for spirometry and skin-prick test (SPT). Maternal and neonatal plasma selenium and erythrocyte GPx were related to the childhood outcomes of wheezing, and asthma.

Results At 2 years 1282 children were followed up. At 5 years symptom data were available for 1167 children, 700 children were SPT tested, and forced expiratory volume in 1 s (FEV₁) was measured in 478. Maternal plasma selenium concentration during early pregnancy was inversely associated with wheezing (odds ratio per 10 µg/kg plasma selenium 0.86, 95% confidence interval 0.76–0.97), and consulting a doctor because of wheeze (0.79, 0.69–0.93) in the second year of life. Cord plasma selenium was also inversely associated with wheezing (0.67, 0.47–0.96), and consulting a doctor because of wheeze (0.62, 0.41–0.93) in the second year of life. By age 5 these associations had disappeared. Maternal and neonatal erythrocyte GPx concentrations were not associated with any childhood outcomes at 2 or 5 years.

Conclusion The selenium status of mothers during early pregnancy, and neonates is associated with early childhood wheezing but not asthma or atopic sensitization, furthermore, this association is absent by the age of 5 years.

Keywords children, glutathione peroxidase, pregnancy, selenium, wheeze

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Introduction

The trace element selenium is a micronutrient essential for human health that could potentially be involved in the pathogenesis and increasing prevalence of asthma because of previously described influences on the immune system [1, 2]. European dietary selenium intake and blood selenium concentrations have declined in recent decades, probably because of the use of selenium-deficient European grain and changes in bread-making technology [2]. In the United Kingdom, the reference nutrient intake for selenium is 75 µg/day for men and 60 µg/day for women

[3]. In 1975 UK average selenium intake was 60 µg/day; but this has declined, current average dietary selenium intake being 30–40 µg/day [2, 4, 5]. Selenium has antioxidant properties because it is incorporated into glutathione peroxidase (GPx) enzymes [1, 6]. Declining dietary antioxidant intake has been proposed as one of the environmental changes that have contributed to the recent increase in asthma [7–9] and several studies have reported associations between asthma and selenium status in adults and children [10–20].

It is becoming increasingly apparent that early life factors are important in the development of asthma. We

have established a cohort of children, recruited *in utero*, to test the hypothesis that maternal antioxidant intake during pregnancy modifies susceptibility to childhood asthma. Along with others we have reported a number of beneficial associations between maternal nutrient intake during pregnancy and asthma and wheezing illness in children up to the age of 5 years [21–24]. Recently, low umbilical cord selenium concentration has been reported to be associated with an increased likelihood of persistent childhood wheezing at 0–6 months and 30–42 months; however because of the large number of analyses performed the authors could not exclude the possibility that the association may have been a chance finding [25]. Using our cohort we have investigated whether wheeze and asthma in children up to 5 years of age is associated with plasma selenium and erythrocyte GPx concentrations in pregnant mothers and in neonates.

Materials and methods

Study subjects and protocol

Full details of recruitment have been described previously [22, 23]. Two thousand healthy pregnant women attending an antenatal clinic, at median 12 weeks gestation, were recruited over 19 months. There was no selection for asthma or atopic disease and apart from expected slight biases (slightly older, less likely to smoke, and of higher socio-economic status) the recruited women were representative of the local obstetric population [22]. At enrolment, an interviewer administered a questionnaire to the women and atopic status was ascertained by skin prick test (SPT). A non-fasting venous blood sample was obtained and the erythrocytes and plasma stored at -70°C . A total of 1924 singletons were born to the 2000 recruited women. Cord blood samples were collected, whenever possible, by the attending midwife within 30 min of delivery, and the erythrocytes and plasma stored at -70°C .

Follow-up

At 1, 2 and 5 years questionnaires based on the ISAAC questionnaire [26] were mailed to all participating families, with up to two reminders [22, 23]. At 5 years, parents were also invited to bring the study child to the hospital for an assessment that included spirometry, bronchodilator response, SPT and measurement of exhaled nitric oxide (FE_{NO}). Bronchodilator response was not included in the original study protocol but was introduced for the last 510 children. Measurements of FE_{NO} were not included in the original study protocol but were included in the last 262 assessments. Details of SPT and the measurement of spirometry, bronchodilator response and FE_{NO} at 5 years have been described previously [23]. The

Grampian Research Ethics Committee approved the study and written parental consent was obtained.

Selenium and glutathione peroxidase assays

Plasma selenium concentration was assayed by inductively coupled plasma-mass spectrometry (ICP-MS) [27]. Plasma samples were diluted 15-fold with an alkali modifier and wetting agent to reduce viscosity and make the samples more amenable to analysis. Owing to the viscous nature of the plasma, all dilutions were carried out on a weight rather than volume basis and all results are reported on a weight/weight basis. An internal germanium-based standard was used to monitor plasma stability in the ICP-MS. A range of selenium standards was made up and used to standardize the ICP-MS. A certified reference material (CRM) Seronorm level 1 MI0181 (Glen Spectra Reference Materials, Middlesex, UK) was prepared in accordance with the supplier's instructions along with the samples and diluted as above. This CRM was analysed with at least every 30 plasma samples to check the performance of the method. All the samples were analysed using an Agilent 7500i ICP-MS with shield torch system (Agilent Technologies, Stockport, UK).

Erythrocyte GPx concentration was assayed using a commercially available enzymatic kit (RS506, Randox Laboratories Inc Co., Antrim, UK) in conjunction with a Roche Cobas Mira Centrifugal Analyser (Hoffmann-La Roche, Basel, Switzerland) [28]. GPx was expressed as units per gram of haemoglobin.

Statistical analysis

The primary outcome variables of interest were the prevalence of wheeze and doctor confirmed asthma in the children at 2 and 5 years of age. Previous work with this cohort has demonstrated associations between maternal nutrient intake during pregnancy and 2 and 5 year outcomes in the children, but not with first year outcomes [21–23]. The main questionnaire-derived outcomes of interest were 'Has your child had wheezing in the chest in the last 12 months', 'Has your child ever suffered from asthma' and 'Has this been confirmed by a doctor'. Secondary outcomes of interest were derived from similar questions asking about eczema and doctor confirmed eczema. The primary exposure of interest was maternal plasma selenium concentration at 12 weeks gestation. Erythrocyte GPx concentration at 12 weeks gestation was analysed as a secondary measure of maternal selenium status. Umbilical cord plasma selenium and erythrocyte GPx were also analysed as secondary exposures of interest.

Based on the observed mean maternal plasma selenium of $80.5\text{ }\mu\text{g/kg}$ (SD 16.6), the study had 80% power at the 5% level of significance to demonstrate a difference of 4% ($3.2\text{ }\mu\text{g/kg}$) in the plasma selenium of mothers of children

with, and without wheeze at 2 years. The corresponding difference for asthma at 2 years was 7% (5.6 µg/kg). At 5 years the study had 80% power at the 5% level of significance to demonstrate wheeze and asthma to be associated with at least 5% (4.0 µg/kg) differences in maternal plasma selenium when compared with children who did not wheeze or have asthma.

Univariate associations between wheeze, and asthma outcomes and demographic, neonatal measurements, nutrients and early life exposures were assessed with Mantel-Haenszel odds ratios. A literature review indicated that the following variables have been associated with wheeze, and asthma outcomes: infant gender, maternal atopy, maternal smoking, maternal age, maternal parity, father's social class, maternal age when left full time education, birth weight, birth crown-heel length, birth occipito-frontal head circumference, maternal (or cord) plasma ascorbate, α -tocopherol and zinc, use of antibiotics during infancy and breastfeeding. The Scottish Index of Multiple Deprivation (SIMD) was used as an indicator of household socio-economic status. SIMD comprises 37 indicators in seven domains and is presented at data zone level, enabling small pockets of deprivation to be identified [29]. Variables with $P < 0.25$ in the univariate analyses were then entered into an ordinary least squared multivariate logistic regression analysis using forced choice selection. The goodness of fit of final models and their ability to discriminate were assessed using Hosmer and Lemeshow goodness of fit tests and the area under the receiver operator characteristic (ROC) curve. To facilitate interpretation, derived odds ratios were expressed in units of 10 µg/kg for selenium and 10 U/gHb for erythrocyte GPx. Analyses were done using SPSS v13.0 (SPSS Inc., Chicago, IL, USA).

Results

Subjects

Plasma selenium and erythrocyte GPx measurements were available for 1867 of the 2000 recruited pregnant women. 1924 singletons were born to the cohort and the questionnaire response rates at 1, 2 and 5 years were 78.6%, 71.4% and 65.1%, respectively. Maternal plasma selenium and erythrocyte GPx measurements were available for 1412 (93.4%), 1282 (93.4%) and 1167 (93.2%) of the children for whom follow-up questionnaire data were available at 1, 2 and 5 years, respectively. One thousand and thirty-six mothers for whom plasma selenium and erythrocyte GPx data were available responded at 2 and 5 years, 131 responded at 5 years only and 246 responded only at 2 years. For children with follow-up questionnaire data at 2 and 5 years, cord plasma selenium measurements were available for 489 (35.6%) and 441 (35.2%),

respectively, and cord erythrocyte GPx measurements were available for 657 (47.8%) and 602 (48.0%), respectively.

At 5 years 797 (64%) children attended the hospital for assessment. All attempted to perform spirometry and 478 children were able to provide a pre-bronchodilator forced expiratory volume in 1 s (FEV₁) measurement. Five hundred and two children attempted post-bronchodilator spirometry and 269 (54%) children were able to provide a post-bronchodilator measurement. Skin prick reactivity and FE_{NO} were determined in 700 and 167 children, respectively.

Tables 1 and 2 demonstrate that the mothers who had plasma selenium measurements and responded to the 2 or 5 year questionnaires were less likely to smoke, were older, of higher socio-economic status, less likely to have wheezed or to have had asthma, and their plasma selenium levels were higher than women who failed to respond. Erythrocyte GPx concentrations did not differ significantly between responding and non-responding mothers. In this population, lower socio-economic status and smoking were associated with reduced plasma selenium and erythrocyte GPx at recruitment. The characteristics of the mothers responding at 2 and 5 years were very similar. The participating 5-year-old children were slightly larger at birth and more likely to have been breastfed and delivered by caesarean section. The mothers and children who attended for hospital assessment were broadly representative of those responding to the questionnaire and while a few significant differences were present these were of small magnitude [23]. The children who were SPT tested, or who had successful measurements of FE_{NO} and FEV₁ were representative of the children attending for hospital assessment [23].

Mean cord plasma selenium concentration was 50.8 µg/kg, [95% confidence interval (CI) 49.7–51.9] and mean cord erythrocyte GPx was 26.9 U/gHb (95% CI 26.1–27.7). Analyses comparing the characteristics of mothers and children by whether cord blood samples were obtained demonstrated similar trends and differences as those reported for maternal plasma selenium and erythrocyte GPx. In contrast to maternal plasma selenium, cord plasma selenium and erythrocyte GPx was associated with the type of delivery, for example mean cord plasma selenium for vaginal deliveries was 51.4 µg/kg, (95% CI 49.6–53.1), for caesarean section 48.3 µg/kg, (46.7–50.0), and for breech deliveries 60.6 µg/kg, (50.5–70.7). Multivariate analyses that included cord selenium and GPx therefore adjusted for type of delivery.

Associations with maternal plasma selenium and erythrocyte GPx

Maternal erythrocyte GPx concentration at 12 weeks gestation was weakly positively associated with plasma

Table 1. Profiles of the mothers on whom plasma selenium and erythrocyte glutathione peroxidase (GPx) concentrations were measured

Maternal characteristic	All mothers of singleton child (<i>n</i> = 1924)	Maternal selenium/GPx and responders at 2 years (<i>n</i> = 1282)	<i>P</i> *	Maternal selenium/GPx and responders at 5 years (<i>n</i> = 1167)	<i>P</i> **
Maternal age recruitment (mean, 95% CI)	28.9 (28.6–29.1)	29.9 (29.6–30.2)	<0.001	29.9 (29.7–30.2)	<0.001
Current smoker at recruitment	566 (29.4%)	297 (23.2%)	<0.001	269 (23.1%)	<0.001
Partner's social class % non-manual	1119 (58.2%)	788 (61.5%)	<0.001	715 (61.3%)	<0.001
Ever had asthma	316 (16.4%)	196 (15.3%)	0.056	176 (15.1%)	0.047
Atopic	689 (35.8%)	473 (36.9%)	0.17	414 (35.5%)	0.69
First pregnancy	656 (35.8%)	437 (35.5%)	0.65	404 (36.8%)	0.84
Dietary supplement use (%)	44.7%	46.0%	0.15	45.6%	0.24
Median plasma selenium µg/kg (5th–95th centile)	79.1 (57.2–107.9)	79.7 (58.2–107.8)	0.015	80.1 (58.8–108.1)	<0.001
Mean plasma selenium µg/kg (95% CI)	80.5 (79.7–81.2)	81.1 (80.1–82.0)	0.014	81.5 (80.5–82.4)	<0.001
Median erythrocyte GPx U/gHb (5th–95th centile)	54.3 (31.0–91.9)	54.7 (31.8–91.8)	0.29	54.3 (31.2–91.6)	0.77
Mean erythrocyte GPx U/gHb (95% CI)	57.1 (56.2–57.9)	57.4 (56.3–58.4)	0.58	56.9 (55.8–57.9)	0.48

**P*-value: responders at 2 years and maternal plasma selenium/GPx vs. non-responders at 2 years and/or no maternal plasma selenium/GPx.

***P*-value: responders at 5 years and maternal plasma selenium/GPx vs. non-responders at 5 years and/or no maternal plasma selenium/GPx. CI, confidence interval.

Table 2. Profiles of the children for whom maternal plasma selenium and erythrocyte glutathione peroxidase (GPx) concentrations were available

Infant variable	All children (<i>n</i> = 1924)	Maternal selenium/GPx and responders at 2 years (<i>n</i> = 1282)	<i>P</i> *	Maternal selenium/GPx and responders at 5 years (<i>n</i> = 1167)	<i>P</i> **
Male	968 (50.3%)	649 (50.6%)	0.70	583 (50.0%)	0.69
Birth weight (g) (mean 95% CI)	3436 (3411–3462)	3481 (3450–3511)	<0.001	3466 (3433–3499)	0.004
Crown-heel length (cm) (mean 95% CI)	49.8 (49.7–50.0)	50.0 (49.9–50.2)	<0.001	50.0 (49.8–50.1)	0.002
Head circumference (cm) (mean 95% CI)	34.8 (34.7–34.9)	34.9 (34.8–35.0)	0.001	34.9 (34.8–35.0)	0.027
Caesarean section (%)	21.7%	22.9%	0.055	22.6%	0.21
Ever breastfed	70.9%	72.7%	0.004	74.4%	<0.001
Breastfed >4months	57.4%	59.8%	0.004	62.2%	<0.001

**P*-value: responders at 2 years and maternal plasma selenium/GPx vs. non-responders at 2 years and/or no maternal plasma selenium/GPx.

***P*-value: responders at 5 years and maternal plasma selenium/GPx vs. non-responders at 5 years and/or no maternal plasma selenium/GPx. CI, confidence interval.

selenium concentrations (Pearson's correlation coefficient 0.12, $P < 0.001$) but was not associated with any childhood outcomes at 2 or 5 years of life. Expression of maternal erythrocyte GPx concentration as quintiles did not reveal any associations with maternal erythrocyte GPx activity in those children born to mothers with suboptimal maternal erythrocyte GPx activity.

Maternal plasma selenium concentration during pregnancy was not associated with any respiratory symptoms during the first year of life.

Maternal plasma selenium concentration during early pregnancy was negatively associated with wheezing, and seeing a family doctor because of wheezing, in the second year of life (Table 3). There were no associations between maternal plasma selenium and asthma in 2-year-olds. Comparisons of mothers with early pregnancy plasma selenium concentrations at the 5th and 95th centiles suggest that 1- to 2-year-old children born to a 5th centile woman, would be 2.36 (2.33–2.38) times more likely to wheeze than children born to a 95th centile woman. There

were no interactions between plasma selenium and maternal smoking, atopy or indicators of socio-economic status. Inclusion of plasma selenium expressed as quintiles indicated that the association between plasma selenium and outcomes was linear with no threshold effect. Logistic regression models using forward and backward stepwise techniques also identified significant associations between plasma selenium and the two year outcomes of wheeze ($P = 0.012$) and seeing a doctor because of wheeze ($P = 0.005$). The final minimal models also included maternal atopy, infant use of antibiotics, birth-weight, gender and SIMD.

There were no associations between maternal plasma selenium during pregnancy and respiratory symptoms, atopic sensitization, ventilatory function or FE_{NO} in the children at 5 years of age. Limitation of the analysis of 2-year outcomes to those mothers who responded at 2 and 5 years did not alter the associations detected between maternal plasma selenium and wheezing outcomes when the children were aged 2 years.

Table 3. Results of uni- and multi-variate analyses of maternal plasma selenium concentration at 12 weeks gestation in relation to childhood outcomes in the second year of life

Outcome during second year	N positive (% of 1282)	OR/10 µg/kg maternal plasma selenium			
		Unadjusted		Adjusted* (n = 1184)	
		OR (95% CI)	P	OR (95% CI)	P
Wheeze in last year	195 (15.2%)	0.85 (0.77–0.95)	0.003	0.86 (0.76–0.97)	0.011
Wheeze in the absence of a cold in last year	77 (5.6%)	0.88 (0.75–1.03)	0.11	0.91 (0.76–1.09)	0.32
Seen by family doctor because of wheeze in last year	151 (11.8%)	0.80 (0.71–0.90)	<0.001	0.79 (0.69–0.93)	0.001
Doctor confirmed asthma	65 (4.7%)	0.88 (0.74–1.04)	0.14	0.92 (0.76–1.12)	0.41

*Adjusted for infant gender, maternal atopy, maternal smoking, maternal age at recruitment, maternal parity, birth weight, birth crown–heel length, birth occipito–frontal head circumference, Scottish Index of Multiple Deprivation, use of antibiotics during first year of child's life and maternal (recruitment) plasma ascorbate.

OR, odds ratio; CI, confidence interval.

Eczema during the first 5 years of life was not associated with maternal plasma selenium or erythrocyte GPx concentrations during pregnancy.

Associations with cord plasma selenium and erythrocyte GPx

Umbilical cord plasma selenium was weakly positively correlated with maternal plasma selenium at 12 weeks gestation (Pearson's correlation coefficient 0.19, $P < 0.001$), but not with cord erythrocyte GPx concentration.

Cord erythrocyte GPx was not associated with any childhood outcomes at 1, 2 or 5 years of life. Cord plasma selenium concentration was not associated with any childhood respiratory outcomes during the first year of life, however like maternal plasma selenium, it was negatively associated with wheezing, and seeing a family doctor because of wheezing, in the second year of life (Table 4). There were no associations between cord selenium and asthma in 2-year-olds. There were no associations between cord plasma selenium and respiratory symptoms, atopic sensitization, ventilatory function or FE_{NO} in the children at the age of 5.

Eczema during the first 5 years of life was not associated with umbilical cord plasma selenium or erythrocyte GPx concentrations.

Discussion

We have demonstrated that low maternal plasma selenium concentration during early pregnancy and probably low cord plasma selenium concentration at birth are associated with an increased likelihood of children wheezing during their second year of life; however, by the age of 5 years these associations had disappeared. In contrast, maternal and neonatal erythrocyte GPx concentrations were not associated with any respiratory outcomes up to the age of 5 years. The association reported here between low cord plasma selenium and increased likelihood of

wheezing outcomes in children during the second year of life is consistent with the report that umbilical cord selenium concentration is negatively associated with persistent childhood wheezing at 0–6 months and 30–42 months [25].

In this cohort we have previously reported that there were no associations between maternal dietary intake of selenium and childhood respiratory outcomes up to the age of 2 years [22]. In that report we highlighted the widely accepted fact that food frequency questionnaire (FFQ) estimates of dietary selenium intake are relatively unreliable. This is because food composition tables, which are used in the estimation of nutrient intake from both FFQs and weighed records, do not capture the wide variation in selenium content of foods due to geographical differences in soil selenium. Indeed, this was the main reason to measure plasma selenium in stored plasma samples. In the present study maternal and neonatal selenium status was assessed by measurement of plasma selenium, in addition, the more easily measured erythrocyte GPx was assayed. Erythrocyte GPx is frequently used as a measure of longer-term selenium status because the GPx activity in the cells reflects selenium intake over the lifespan of the erythrocyte (120 days) [30]. Erythrocyte GPx activity correlates with tissue selenium status up to certain levels, then activity plateaus, even though tissue selenium content can continue to rise. Therefore in a population such as reported here, with a maternal plasma selenium of approximately 1 µM (79 µg/L) or greater, the optimal way of detecting increased selenium intake is to measure plasma levels of the element itself. Plasma GPx activity is more sensitive to short-term changes in selenium intake and is useful in supplementation trials. However, plasma GPx activity plateaus and does not increase further if plasma selenium rises above approximately 1 µM (79 µg/L) [30].

In the present study the mean plasma selenium concentration in this population of pregnant women at 12 weeks gestation was 80.5 µg/kg (95% CI, 79.7–81.2). This

Table 4. Results of uni- and multi-variate analyses of neonatal cord plasma selenium concentration in relation to childhood outcomes in the second year of life

Outcome during second year	N positive (% of 489)	OR/10 µg/kg cord plasma selenium			
		Unadjusted		Adjusted* (n = 1061)	
		OR (95% CI)	P	OR (95% CI)	P
Wheeze in last year	63 (12.9%)	0.71 (0.55–0.93)	0.012	0.67 (0.47–0.96)	0.030
Wheeze in the absence of a cold in last year	27 (5.5%)	1.018 (0.78–1.33)	0.90	0.978 (0.47–1.30)	0.35
Seen by family doctor because of wheeze in last year	49 (10.0%)	0.71 (0.51–0.99)	0.043	0.62 (0.41–0.93)	0.022
Doctor confirmed asthma	22 (4.5%)	0.97 (0.70–1.34)	0.84	0.95 (0.57–1.58)	0.85

*Adjusted for infant gender, maternal atopy, maternal smoking, maternal age at recruitment, maternal parity, mode of delivery, birth weight, birth crown-heel length, birth occipito-frontal head circumference, Scottish Index of Multiple Deprivation use of antibiotics during first year of child's life and cord plasma ascorbate.

OR, odds ratio; CI, confidence interval.

is very similar to a mean plasma selenium of 80.0 µg/L, (76.2–83.8) reported by a previous unrelated study of 45 local healthy non-smoking men and women aged 25–50 years [31]. The mean maternal plasma selenium observed in this study is probably less than the rest of Scotland, England and Wales because The National Diet and Nutrition Survey of 1347 men and women aged 19–64 years reported a mean plasma selenium for participants from Scotland of 84 µg/L, with mean plasma selenium concentrations for participants from Northern England, Central/South-West England/Wales and London/South-East England being 85, 84 and 92 µg/L, respectively [32]. The mean maternal erythrocyte GPx concentration of 57.1 U/gHb (56.2–57.9) may be less than that reported locally in healthy men and women (62 U/gHb, 57.3–66.7) [31], however erythrocyte GPx has been reported to decline in the first trimester of pregnancy, whereas plasma selenium declines in the second trimester [33].

The recruited study population was very similar in demographic and obstetric characteristics to the local obstetric population [22, 23], and the mothers for whom plasma selenium and erythrocyte GPx estimations were available at recruitment were representative of the study population. The mothers responding at 2 and 5 years were very similar, however, when compared with non-responders they were more likely to be non-smokers and have higher socio-economic status and plasma selenium at recruitment. It is likely that non-respondent children were more likely to wheeze because an analysis of the wheezing symptoms of the children participating at 1 year but not at 2 years indicated that the children not participating at 2 years were more likely to have wheezed by 1 year (not presented). This type of response bias often plays a role in cohort studies because it is known that subjects with a poorer social economic status and life-style (lower educational level, poorer diet, smoking, etc.) are more difficult to trace, and that people who suffer poor health during the follow-up period are prone to attrition [34–37]. While there was evidence of typical response biases

among those participating in this study, it is likely that the observed associations in this study are underestimated rather than overestimated, for instance improved ascertainment at 2 years would have resulted in a larger proportion of wheezy children with low maternal plasma selenium concentrations, making the observed associations between maternal plasma selenium and childhood wheezing symptoms stronger. While it is possible that the associations reported here could be chance associations consequent upon multiple testing, we feel that this is unlikely. Firstly, the number of primary comparisons was deliberately limited (eight in total: four outcomes at 2 and at 5 years, one exposure: maternal plasma selenium) and after Bonferroni correction of the primary analyses the association between plasma selenium and consulting the family doctor because of wheeze in the second year of life remained significant (adjusted $P=0.008$). Secondly, the associations with maternal and neonatal plasma selenium and outcomes in 2 year children were similar. Thirdly, the associations reported here are consistent with a recent report that umbilical cord selenium is negatively associated with persistent wheezing between 0–6 and 36–42 months [25]. We are aware of only two studies (including this one) relating maternal selenium status during pregnancy to childhood respiratory disease and it is unlikely that both of these will report very similar associations by chance. The issue of chance associations being reported by two studies can only be addressed by further studies. In this cohort we have previously reported associations between maternal intake of vitamin E, vitamin D and zinc during pregnancy and wheezing outcomes in children at 2 and 5 years of age [21–23]. The associations between maternal and cord plasma selenium and wheezing outcomes in 2-year-old children are unlikely to be the consequence of confounding by these nutrients because their inclusion, instead of blood levels, in the multivariate analyses did not appreciably alter the magnitude or significance of the associations between plasma selenium and wheezing outcomes in 2-year-old children, for

example, for maternal plasma selenium and wheeze in the second year of life, after inclusion of variables outlined in Table 3 and maternal vitamin C, vitamin E, zinc and vitamin D intakes, odds ratio 0.87 (0.78–0.97), $P=0.014$. Although we adjusted for variables linked to socio-economic status, we cannot entirely eliminate the possibility that the observed associations between selenium and early childhood wheeze are a consequence of residual confounding by factors associated socio-economic status. The absence of associations between maternal plasma selenium and outcomes in 5-year-old children is unlikely to be a consequence of reduced power or response bias because, power was similar between the two study phases and the associations with 2-year outcomes persisted when the analyses were limited to the women who responded at 2 and 5 years. We have hypothesized that the recent increase in asthma is, in part, a consequence of the westernized diet becoming increasingly deficient in antioxidants, resulting in increased population susceptibility to asthma [7]. Several studies have reported associations between asthma and selenium. In children, reduced serum selenium has been associated with asthma [10] and wheeze 8 years later [11]. In adults, reduced serum, urine and dietary selenium have been associated with asthma [12–16], and serum selenium has been positively associated with FEV₁ [17]. Fewer studies have reported associations between asthma and GPx [13, 16, 19]. There are two potential immunological mechanisms whereby early life selenium status could influence the likelihood of early childhood wheezing. Reduced maternal and/or neonatal plasma selenium status has the potential to modify the likelihood of childhood asthma and atopic disease by influencing the first interactions between allergens and the immune system. There is some data suggesting that low selenium status promotes T-helper (Th)-cell differentiation towards the Th2 phenotype associated with asthma and atopic disease [38–40]. A potential explanation for the transient associations reported is that any association with asthma associated wheeze in the first year of life is masked by the predominance of viral associated wheeze and that any effect of maternal selenium status on childhood wheezing is short lived and superseded, as the children develop their own selenium status dependent on their dietary selenium intake. Such a mechanism predicts that reduced maternal and/or neonatal plasma selenium status should be associated with an increased likelihood of early childhood eczema. Clearly, this was not the case in the present study, suggesting that any link between wheeze and selenium, if causal, is not primarily operating through effects on Th2 differentiation and atopy.

The demonstration that selenium status modifies the immune response to, and the handling of, viral infections provides a second possible mechanism by which early life selenium status could influence the likelihood of early life wheeze but not the subsequent development of asthma

and atopic disease. Wheeze in the preschool years is often transient, viral associated and independent of atopy [41]. Broome et al. [40] reported that in human volunteers suboptimal selenium status is associated with increased replication and delayed clearance of live attenuated polio vaccine because of reduced and delayed CD3⁺ T lymphocyte proliferative and interferon- γ responses. It is possible therefore that reduced maternal and/or neonatal plasma selenium status impairs the ability of child's immune system to mount an effective response to viral infection, resulting in an increased risk of severe protracted respiratory symptoms and medical consultation. The transient associations reported at 2, but not 5 years in the present study are more in keeping with an effect of early life selenium status on early childhood viral associated wheeze rather than an effect on Th2 immune differentiation.

In the present study maternal erythrocyte GPx was not associated with any childhood respiratory or atopic outcomes. In this study population, mean maternal plasma selenium was 80.5 $\mu\text{g/kg}$ making it likely that over half of the women will have had optimal GPx activity, however, there was no evidence of an association between maternal erythrocyte GPx and childhood outcomes in the 670 children of mothers with suboptimal erythrocyte GPx activity (plasma selenium < 80.5 $\mu\text{g/kg}$). The absence of any associations between erythrocyte GPx and childhood outcomes suggests that the associations between plasma selenium and early childhood wheeze are mediated by mechanisms independent of GPx, which is one of many selenoproteins. In addition, it is recognized that selenium can act independently of GPx and selenoproteins by directly affecting cell metabolism [42].

We suggest that while the recent decline in dietary selenium intake may have impacted on early childhood respiratory health, any antenatal effect of maternal selenium status during pregnancy is absent by the age of 5 years and not translated into diagnosed asthma or to the development of atopy.

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