Maternal vitamin D and E intakes in pregnancy and asthma to age fifteen years: a cohort study

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Running head: Maternal diet and childhood asthma

Abstract

This study investigated whether the previously reported associations in this birth cohort between maternal vitamin D and E intakes during pregnancy and childhood wheeze/asthma outcomes at age five and ten years are still evident at age fifteen years.

In a prospective study of 1924 children recruited *in utero*, maternal vitamin D and E intakes during pregnancy were assessed by food frequency questionnaire and the children completed respiratory questionnaires at age fifteen years. Treatment for asthma at age fifteen was also ascertained using healthcare data. Maternal vitamin D and E intakes were also related to combined childhood wheeze/asthma data collected at one, two, five, ten and fifteen years of age.

Symptom data were available for 747 (39%) fifteen-year olds and healthcare data for 1689 (88%). There were no associations between maternal vitamin D and E intakes and childhood wheeze and asthma outcomes at age fifteen. Analysis of combined data collected between one and fifteen years of age demonstrated that higher maternal vitamin D and E intakes during pregnancy were associated with a reduced likelihood of being diagnosed with asthma in the first fifteen years: hazard ratio (95% CI) per quartile increase in vitamin intake of 0.87 (0.78,0.98) and 0.88 (0.78,0.98) respectively.

Lower maternal vitamin D and E intakes during pregnancy are associated with increased risk of children wheezing and being diagnosed with asthma in the first ten years but not after puberty, suggesting that post-natal exposures predominate in the aetiology of incident asthma as children transition through puberty into adulthood.

Word count n=249

Introduction

Asthma continues to be a major health issue. Globally 300 million people have asthma and as societies transition to a modern urban lifestyle, by 2025, an additional 100 million people will have asthma[1]. The overall disease burden of asthma remains high for those affected and healthcare systems. Investigation into asthma causation remains a key research priority.

It has been hypothesised that changing diet contributed to the increase in asthma observed in economically developed countries in the latter decades of the 20th century[2]. Subsequent studies have related maternal nutrient intake/status during pregnancy to the development of asthma and wheeze in children[3-6]. Whilst the majority of studies have been observational, some interventional trials have been reported[7,8]. Systematic reviews have identified higher maternal vitamin D and E intake/status during pregnancy to be associated with a reduced likelihood of wheezing illness in children[3-6].

The SEATON (Study of eczema and asthma to observe effects of nutrition) birth cohort was recruited *in utero* to identify associations between maternal diet during pregnancy and childhood asthma and atopic disease. The cohort has been followed up at age one, two, five and ten years and was one of the first to report associations between lower maternal vitamin D and E intakes during pregnancy and an increased likelihood of childhood wheeze and asthma up to the age of ten years[9-12].

We present here the results of the fifteen year cohort follow-up, performed to test the hypothesis that the associations between wheeze/asthma outcomes at age five and ten years with maternal vitamin D and E intakes during pregnancy persist beyond puberty. We believe this to be one of very few birth cohorts repeatedly followed-up in order to prospectively relate maternal nutrient intakes during pregnancy to wheeze outcomes throughout childhood and beyond puberty.

Methods

The current follow-up replicated the methodology described in previous phases of follow-up of this birth cohort: the fifteen year follow up comprised a postal questionnaire, detailed clinical assessment and use of routine healthcare data. The wheeze and asthma questionnaire data collected at the one, two, five, ten and fifteen year follow-ups were combined for longitudinal analysis[9-12]. Maternal vitamin D and E intakes were then related to wheeze/asthma outcomes at fifteen years and to the combined one, two, five, ten and fifteen year data.

Recruitment of pregnant women.

Between 1997 and 1999, 2000 healthy unselected pregnant women attending routine antenatal clinics were recruited at median 11 weeks gestation[9]. At enrolment, an interviewer administered a questionnaire, allergen skin prick testing (SPT) was performed and a non-fasting blood sample obtained. Plasma α-tocopherol was quantified by normal phase high-performance liquid chromatography. At 32 weeks gestation, dietary intake during the preceding three months was assessed using version 5.4 of the Scottish Collaborative Group Food Frequency Questionnaire (FFQ)[13]. In women of childbearing age the correlation coefficients between 4-day weighed records and FFQ derived vitamin D and E intakes were 0.38 ($p<0.001$) and 0.52 ($p<0.001$) respectively[14].

Assessment of children at age fifteen

Singletons born to recruited women were invited to participate at age fifteen. An ISAAC based questionnaire, was mailed to cohort children in the month of their 15th birthday, with a single reminder if necessary[9,11]. Wheeze was defined by an affirmative response to the question "have you had wheezing or whistling in the chest in the past 12months?" Similar questions enquired about "ever wheezed," and "wheezed in the absence of a cold." Doctor-diagnosed asthma (DDA) was defined by positive responses to the questions "have you ever suffered from asthma?" and "was this confirmed by a doctor?" Use of asthma medication/inhalers in the last year was also ascertained. Current asthma was defined as asthma and wheeze in the previous year. Similar questions enquired about eczema and hayfever.

Children responding to the questionnaire were invited to complete an FFQ assessing dietary intake[13] and to attend an assessment that included spirometry and SPT[11,12,15].

The North of Scotland Research Ethics Committee (13/NS/0108) approved the study, written parental consent was obtained, and children gave written assent.

Healthcare data follow-up

In the UK, asthma medications are only available by prescription and in Scotland individual patient level dispensing information for >98% of General Practitioner prescriptions is retained in the Prescribing Information System (PIS) database maintained by NHS Scotland[16]. PIS was used to increase case ascertainment for the fifteen year outcome "have you been prescribed medicines/inhalers for asthma in the last 12months?" PIS was used to identify cohort children resident in Scotland and whether they had been dispensed asthma medication (British

National Formulary codes 3.1–3.3[17]) ≥2 occasions in their fourteenth year. The use of PIS and the linkage of asthma medication to the main study database in a secure data haven, followed by immediate anonymization by an independent third party was approved by the Public Benefit and Privacy Panel for Health and Social Care.

Statistical analysis

Asthma and wheeze outcomes at age fifteen were related to maternal plasma α-tocopherol, vitamin D and E intakes. For women completing the FFQ, dietary and supplement intakes were summated, energy-adjusted and divided into quarters[18]. Plasma α-tocopherol was logarithmically transformed, adjusted for cholesterol and gestational age, and presented per standard deviation increase. Unadjusted and adjusted modelling was performed: adjusted models included potentially confounding covariates selected on: conceptual evidence (maternal smoking, maternal atopy (≥1 positive SPT, 3mm cutoff), birth order, child's sex, maternal age at recruitment and maternal socio-economic status (SES) (Scottish Index of Multiple Deprivation (SIMD)[19], and statistical tests (birth weight, length, head circumference), achieving $p<0.25$ in univariate analyses[20]. Associations with vitamin D were adjusted for vitamin E and vice versa. To identify additive associations, energy-adjusted maternal vitamin D and E intakes were dichotomised about the median value into 'high' and 'low' intakes and coded as D^{high}/E^{high}, D^{high}/E^{low}, D^{low}/E^{high} and D^{low}/E^{low}. Maternal and childhood vitamin D and vitamin E intakes were similarly combined and coded. Further analyses included children's nutrient intake at age fifteen, combined maternal-childhood nutrient intakes and replaced area based SES parameters with alternative metrics based on parental occupation and maternal education. Maternal nutrient intake and status was related to fifteen-year

childhood wheeze and asthma outcomes using logistic regression. For longitudinal analysis, data collected at fifteen years were combined with data collected at one (n=1511), two (n=1373), five (n=1253) and ten years (n=934). Generalized estimating equations were used to relate maternal nutrient intake/status and repeated (and correlated) measurements of childhood 'wheeze in the last year' and 'current asthma' ascertained at ages one, two, five, ten and fifteen years. Cox proportional hazard modelling was used to relate maternal nutrient intake/status to the age when doctor diagnosed asthma was first reported. Analyses were performed using IBM SPSS Statistics for Windows, v24.0 (Armonk, NY).

Results

There were 1924 live singleton children born to the 2000 recruited pregnant women, but because of withdrawals questionnaires were sent to 1863 children at age fifteen. Questionnaires were returned for 747 (39% of 1924). Maternal dietary and α-tocopherol data during pregnancy were available for 724 (95%) and 747 (100%) children respectively. The children's FFQ was completed by 557, and 539 attended clinical assessment; 515 performed spirometry, and 529 underwent SPT. The PIS based follow-up comprised 1689 (88%) children; asthma medication use data were available for 1656 children using PIS and for an additional 33 who returned questionnaires from outwith Scotland. The 235 children for whom there were no PIS data comprised 61 for whom consent had been explicitly withdrawn and 174 who lived outwith Scotland. Figure 1 details the women/children participating in the longitudinal study.

Comparing with characteristics at recruitment and at birth, the mothers of children participating in the questionnaire and clinical phases were less likely to smoke, were older, of higher SES, less likely to have wheezed and had higher plasma ascorbate and β-carotene concentrations, whereas participating children were more likely to be girls and of higher birth weight (Tables 1, online supporting information E-table 1). Participation was not differentially associated with maternal vitamin D, maternal asthma, atopy, or plasma α-tocopherol. The mothers of children participating in the clinical assessment had lower vitamin E intake during pregnancy. Although numbers were small, the mothers of children not included in the PIS follow-up (consent withdrawn or living outwith Scotland) were less likely to smoke or be

asthmatic, had higher vitamin D intakes, higher plasma α-tocopherol and tended to be of higher SES.

Table 2 details the prevalence of wheezing, asthma, eczema and hayfever in the fifteen-year-old children. Use of asthma medication in the last year was reported by 94 (12.8%) of questionnaire respondents and by 170 (10.1%) using PIS follow-up. Paired PIS and questionnaire data were available for 730 children, 75 were treated for asthma in both questionnaire and PIS, whereas 627 were not treated for asthma in both. PIS with respect to questionnaire had sensitivity 80%, specificity 99%, positive predictive value 89%, negative predictive value 97% and Kappa 0.82.

Of the 1924 cohort children 253 (13.1%) never participated in any of the follow-ups, 180 (9.4%) participated once, 236 (12.3%) twice, 370 (19.2%) on three occasions, 371 (19.3%) four times and 514 (26.7%) participated in all five follow-up phases. Of the 565 children participating at age ten and fifteen, 41 (7.3%) reported asthma at ten and fifteen, 27 (4.8%) reported asthma at fifteen but not ten and 21 (3.7%) reported asthma at ten but not fifteen. At some point in fifteen years of follow-up 414 (21.5%) of the original 1924 children had reported 'wheeze in the last year' and 285 (14.8%) had reported DDA.

Analyses of 724 children with fifteen year questionnaire and maternal FFQ data demonstrated that lower maternal vitamin D intake during pregnancy was associated with an increased likelihood of a report of 'ever wheezed' by fifteen year old children (table 3). There were no

associations between maternal vitamin D or E intakes with asthma or wheeze in the previous 12 months in fifteen year old children (tables 3, 4 and 5). Analysis of the PIS follow-up revealed no associations between use of asthma medication/inhalers in the fifteenth year and maternal vitamin D or E intakes or plasma α-tocopherol during pregnancy. Longitudinal analysis of the combined one, two, five, ten and fifteen year data demonstrated that low maternal vitamin D and E intakes during pregnancy were associated with an increased likelihood of reporting 'wheeze in the last year' and DDA in their first fifteen years. Repeating the analyses excluding birth weight, length, head circumference did not substantially alter the findings (<10% change in odds ratios).

Energy adjusted maternal vitamin D and E intakes were weakly correlated (Pearson's correlation coefficient 0.29, $p<0.001$). Dichotomising maternal vitamin D and E intakes into 'high' and 'low' intakes demonstrated an additive effect. At age fifteen, when compared with children born to women with vitamin D^{low}/E^{low} intakes, children born to women with vitamin D^{high}/E^{high} intakes were less likely to have 'asthma ever' OR 0.52, (95% CI 0.28,0.94) or DDA OR 0.46 (0.25,0.86). In longitudinal analyses, when compared with children born to women with vitamin D^{low}/E^{low} intakes children born to women with vitamin D^{high}/E^{high} intakes were less likely to have reported 'wheeze in the last year' and DDA in their first fifteen years: OR 0.75, (0.59,0.95) and HR 0.65, (0.46-0.91) respectively. Maternal vitamin D or E intakes during pregnancy were not associated with FEV_1, FVC or atopic status in the 539 children attending clinical assessment. Use of non-area based SES metrics or presence of household smokers at age fifteen did not significantly alter any of the analyses. Inclusion of season of birth or season of last menstrual period in the analysis of the maternal vitamin D data did not alter the study

findings. Classification of doctor diagnosed asthma and wheeze outcomes into atopic (based on the results of skin prick tests or history of eczema or hayfever) or non-atopic did not alter the study findings. Dietary FFQ data for 557 fifteen year old children were available; the vitamin D and E intakes of these children were not associated with wheeze, asthma, lung function or atopic outcomes. Maternal and childhood vitamin D and E intakes were very weakly correlated, Pearson correlation coefficient $r = 0.095$, $p=0.027$ for vitamin D and $r = 0.11$, $p = 0.012$ for vitamin E. Combined maternal child vitamin D and E intakes were not associated with any childhood outcomes.

Discussion

We believe this to be one of very few birth cohorts repeatedly followed-up in order to prospectively investigate the natural history of associations between maternal vitamin D and E intakes during pregnancy and childhood wheezing illness. The main findings were inverse relationships between maternal vitamin D and E intakes during pregnancy and wheeze and asthma during the first fifteen years but not at fifteen years. The main implication of the finding is that whilst optimising maternal diet during pregnancy may lessen high burden of respiratory symptoms in young children, it is likely that any benefit to offspring would diminish and then disappear with the passage of time.

The results at age fifteen need to be considered in context of previous follow-up phases of our cohort. Lower maternal vitamin D intake during pregnancy was associated with an increase in childhood wheezing between two and five years[10] and an increase in wheeze and asthma at age ten[12]. Lower maternal vitamin E intake during pregnancy was associated with an increase in childhood wheeze at age two and five and with an increase in asthma at age five[9,11]. Lower maternal plasma α-tocopherol during pregnancy was associated with reduced FEV_1 at age five and an increase in asthma at age ten[11,12]. The current fifteen year follow-up demonstrates no associations between maternal vitamin D or E and current wheeze or asthma outcomes at age fifteen. However, longitudinal analyses demonstrated associations between maternal vitamin D and E intakes and wheeze and asthma outcomes in the first fifteen years of life, supporting the findings of previous phases of follow-up. The most plausible explanation for these observations is that maternal vitamin D and E intakes influence the development of childhood wheeze and asthma up to about the age of ten but

that other (postnatal) exposures predominate in the aetiology of incident asthma as children transition through puberty into adulthood, with some children 'growing out' of their asthma and some developing asthma. The previous phases of follow-up reported no association between the diets' of the children at 5 and 10 years, as measured by age specific FFQ, and asthma and wheeze outcomes[11,12]. Similarly the current phase of follow-up found no association between the diets of the children at age fifteen years and wheeze/asthma outcomes suggesting that maternal diet during pregnancy is more important in the aetiology of wheeze and asthma than childhood diet.

The results of the previous phases of this cohort study are consistent with the findings of several systematic reviews. However, the findings of the current study that any associations with maternal vitamin D and E intakes disappear by the age of fifteen is an important advance in the field by following-up children at an age older than any study included in the systematic reviews. Feng et al and Song et al conducted systematic reviews of birth cohort studies investigating associations between childhood wheeze/asthma and maternal and/or cord blood 25(OH) vitamin D_3[5,6]. Both reviews identified 15-16 studies and reported that when compared with lower 25(OH)D_3 concentrations higher maternal 25(OH)D_3 was associated with reduced risk of childhood asthma and/or wheeze. Both studies found no evidence of a linear association between maternal/cord blood 25(OH)D_3 and childhood wheeze/asthma but instead a U-shaped dose–response relationship was identified[6]. Most of the studies reported follow-up at a single time point. The mean age of the children included in these systematic reviews was 5-6 years with the oldest children being aged fourteen. Zosky et al reported maternal lower maternal 25(OH)D_3 concentrations to be associated with an increased risk of

childhood wheeze at age six but not at fourteen[21]. Although there was an association with childhood asthma at age six, this was in boys only and not evident at age fourteen. When compared with the Zosky et al study, our study is somewhat larger, investigated maternal vitamin D and E intakes (not blood concentrations) and had somewhat stronger associations with asthma and wheeze at five and ten[11,12]. The current study found no evidence at age 15 years of the adverse association between maternal serum 25(OH)D_3 concentrations during pregnancy and asthma related hospitalisation up to the age of 25 years reported by Hansen et al[22].

In their systematic review Beckhaus et al identified five birth cohort studies and reported maternal vitamin D intake during pregnancy to be associated with a reduction in childhood wheeze[3]. The oldest age of follow-up of the included studies was seven years and they reported no associations with asthma. The ten and fifteen year follow-up of the current cohort add to this systematic review by demonstrating associations with asthma at age ten that have waned by age fifteen. The systematic review of Wu et al identified ten studies and reported maternal vitamin E intake during pregnancy to be associated with reduced risk of childhood asthma and wheeze[4]. The oldest age of follow-up of the included studies was ten years, the current study would suggest that these associations are likely to be absent by age fifteen.

The strengths of the current study include extended follow-up of a birth cohort that has previously reported associations between childhood wheeze/asthma and maternal vitamin D and E. A further strength was use of routinely collected healthcare data to increase case

ascertainment to 88%. In addition, outcome data prospectively collected at one, two, five, ten and fifteen years were collated providing more robust measures of potential health effects and insight into natural history than single time point outcomes could provide. These longitudinal analyses enabled inclusion of all available outcome data collected over the years and were not restricted to the minority of children who provided outcome data at all the phases of follow-up (n=514), we did not attempt to relate maternal nutrient status to the various childhood wheezing phenotypes that have been reported in larger birth cohorts. We consider that the substantial changes in the magnitude of the estimates of association between ten and fifteen years represent real changes in association rather than being a consequence of methodological issues, such as drop out or asthma diagnostic uncertainty.

Limitations of the study were that 39% and 28% of the cohort participated in the postal and clinical phases, compromising our ability to confidently comment on associations between maternal vitamin D and E and objective parameters of childhood asthma and atopy and on associations with the diet of the children. Participation was associated with significant biases; however these biases were very similar in direction and magnitude to those observed in previous phases of follow-up and unlikely to produce a type II error masking the findings reported at age ten. An additional limitation is that we did not ascertain the age at which a diagnosis of asthma was made by a doctor, consequently the longitudinal analysis of doctor diagnosed asthma was limited to the age at which a diagnosis of asthma was first reported by participating mothers/children. A further limitation was that our only vitamin D metric was an FFQ estimate that was reliant on maternal recall of foods consumed and of their quantities.

The limitations and measurement errors associated with FFQs are well known; however the FFQ used was sufficient to identify associations at two, five and ten years.

Two recent well conducted randomised placebo-controlled vitamin D intervention trials have reported vitamin D supplementation during pregnancy does not significantly reduce persistent wheezing in children up to the age of three years, although for VDAART the effect size was of borderline significance[7,8]. It is notable that for VDAART most of the beneficial effect of maternal vitamin D supplementation appeared to be in the first year, with absolute effect size declining with age[8]. The results of the current cohort study suggest that any effects of vitamin D supplementation during pregnancy should be evident until age ten. Although they should be considered with a degree of caution because of lack of association in the PIS follow-up, the analyses of combined maternal vitamin D and E intakes demonstrating associations with asthma outcomes at age 15 suggest perhaps, that a multifaceted approach to intervention during pregnancy with several nutrients should be considered.

The genesis of this study was in 1990, when an alarming rise in asthma among schoolchildren had been observed in developed countries. The rise in the UK coincided with a reduction in the nutrient content and/or intake of fresh fruit and vegetables across the population[2,23]. We hypothesised that such a change would increase population airway susceptibility to allergens and irritants and thus contribute to the rise in prevalence[2]. Our early studies pointed to vitamins E and D having a role in airway disease and, since the rise in prevalence had occurred in young children, we proposed that the mother's diet during pregnancy would influence the child's risk of developing asthma[24]. This study, now complete to age fifteen, has confirmed

that there is an association between risk of early childhood asthma and a maternal diet characterised by low intakes of vitamins E and D. As the lungs grow towards puberty, so the effect diminishes and disappears, but leaves behind a trail of family anxiety and health service costs. In 1994 we predicted that improvements in childhood nutrition would reduce the prevalence of childhood asthma[2]. In Aberdeen sequential studies of pregnant women in the same antenatal clinic, using the same FFQ have documented an increase in mean (95% CI) vitamin E intake from 9.41mg/d (9.01-9.83) in 1997/8, to 11.9mg/d (10.4-13.5) in 2009 and to 14.7mg/d (11.7-17.6) in 2014[9,25,26]. Similarly the UK National Diet and Nutrition survey reported that in 2000/1 mean (SD) plasma α-tocopherol in women aged 19-64 years was 16.6 (4.53) – 24.7 (6.70)µmol/l and in 2011/2 32.0µmol/l (9.09)[27,28]. Intriguingly there is now evidence that the prevalence of childhood asthma in the UK is decreasing[29,30].

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Figure Legend

Figure 1: Summary of children involved in the various aspects of the fifteen year follow up.