ON LINE SUPPLEMENT

Maternal intakes of vitamin D and E during pregnancy are associated with childhood asthma at age 10.

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METHODS

Parents responding to the questionnaire were invited to complete an FFQ (version C2 SCG FFQ) to assess the study child's dietary intake over the previous three months.[1] Version C2 is a 140 item semi-quantitative FFQ based on the questionnaire used for the mothers, but modified to be appropriate for children aged 3-11 years.

Parents responding to the postal questionnaire were invited to bring the study child to the hospital for an assessment that included spirometry, methacholine responsiveness, skin prick testing and measurement of exhaled nitric oxide (FE_{NO}).

Spirometry and bronchodilator response

Spirometry was measured using a pneumotachograph (Spirotrac IV version 4.22, Vitalograph, UK) with on-screen incentive software. Spirometric values presented were the best from at least two technically acceptable expiratory manoeuvres where the ratio of back-extrapolated volume to FVC was less than 5%, there was a rapid rise to peak expiratory flow and smooth descent of the flow-volume curve, and forced expiratory time exceeded 0.5 seconds.[2]

Skin prick testing

Skin prick reactivity to the allergens dog, cat, timothy grass, egg, peanut, and house dust mite (ALK Abello, Hungerford, UK) was determined. The negative control was 0.9% saline and the positive control was histamine 10mg/ml. A positive response was defined as a mean weal diameter 3mm or greater than the negative control 15 minutes after inoculation. Atopy was defined as at least one positive response.

Measurements of exhaled nitric oxide

A NIOX® analyser (Aerocrine, Sweden) was used to measure FE_{NO} after spirometry and bronchodilator response. FE_{NO} was measured in accordance with International Guidelines.[3] Up to nine attempts were permitted [4] in order to obtain mean values from either two measurements within 5% or three within 10% of each other.[3]

Methacholine Responsiveness.

A methacholine provocation study was conducted to quantify bronchial hyperresponsiveness.[5] Pharmaceutical quality methacholine solutions were obtained from the Pharmacy Department of Wythenshawe Hospital, Manchester, UK. These had been prepared in sterile conditions, under procedures of quality assurance, under a Medicines and Healthcare products Regulatory Agency (MHRA) license. Solutions were sent ready prepared at required concentrations.

Concentrations of 0.0625, 0.25, 1.0, 4.0, 16.0 mg/ml (representing cumulative doses of 0.003, 0.014, 0.059, 0.239 and 0.959 mg/ml respectively) were nebulised using 5 characterised DeVilbiss 646 nebulisers connected to a KoKo dosimeter, run off medical air at 30 psi. The mode of the dosimeter was set to normal, the dose duration to 0.6 seconds, inhale time to 5 seconds, hold time to 5 seconds, dose count to 5 and series timer to 0.15 minutes.

The child was instructed to inhale slowly and deeply from the nebuliser and to then hold their breath for 5 seconds. Musical cues from the dosimeter prompted the child to hold their breath. This step was repeated until 5 inhalations had been performed in no more than three minutes.

The child's FEV1 was measured 30 and 90 seconds after the last inhalation. Provided that both of these manoeuvres were technically acceptable, the lowest FEV1 was recorded. This was repeated with increasing concentrations of methacholine until the child's FEV1 fell by ≥20% or the highest concentration of methacholine was administered.

The test was deemed to be positive if a child's FEV1 fell by 20% or more of their recorded baseline spirometry. The test was stopped at the occurrence of a positive reaction or once spirometry had been gained after exposure to the final dose of methacholine.

Any bronchoconstriction was reversed by the administration of 400 μg albuterol administered via a large volume spacer and recovery of FEV_1 was confirmed by spirometry 15 minutes later. to assess recovery.

Airway responsiveness was expressed as the dose required to induce a 20% decrease in FEV_1 and as a percentage dose response slope (%decline in FEV_1 post challenge / Total cumulative methacholine dose).

REFERENCES

1. Craig LCA, McNeill G, Macdiarmid JI, Masson LF. Dietary patterns of school-age children in Scotland: association with socio-economic indicators, physical activity and obesity. *Br J Nutr* 2010;103:319-334.

2. Aurora P, Stocks J, Oliver C, Saunders C, Castle R, Chaziparasidis G, Bush A. Quality control for spirometry in preschool children with and without lung disease. *Am J Respir Crit Care Med* 2004;169:1152-9.

3. ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide. *Am J Respir Crit Care Med* 2005;171:912-930.

4. Napier E, Turner SW. Methodological issues related to exhaled nitric oxide measurement in children aged four to six years. *Pediatr Pulmonol* 2005;37:24-30.

5. Guidelines for Methacholine and Exercise Challenge Testing 1999. *Am J Respir Crit Care Med* 2000;161:309-329.

Table E1 Characteristics of mothers at recruitment, and those who brought their child for clinical assessment.

	All mothers of singleton child	Clinical assessment	
	n=1924	n=449	P**
Maternal			
Age at recruitment (median, IQR)	29 (26,33)	31 (24,32)	<0.001
Current smoker at recruitment n(%)	566 (29.4%)	75 (16.7%)	<0.001
SIMD at recruitment Median (IQR)	8.86 (5.30,16.78)	7.21 (5.42,18.6)	<0.001
Age left full time education (Mean, 95% CI)	18.3 (18.1,18.4)	18.8 (18.6,19.1)	<0.001
Ever wheeze n(%)	708 (36.8%)	141 (31.4%)	0.007
Asthma ever n(%)	316 (16.4%)	64 (14.3%)	0.161
Atopy n(%)	689 (35.8%)	168 (37.5%)	0.400
First pregnancy n(%)	656 (35.8%)	154 (36.1%)	0.907
FFQ returned n(%)	1717 (89.2%)	440 (98%)	<0.001
Dietary supplements (%)	44.7%	44.0%	0.733
Vitamin D intake µg/d (GM, 95% CI)	3.60 (3.50,3.71)	3.62 (3.42,3.82)	0.817
Vitamin E intake mg/d (GM, 95% CI)	8.20 (8.10,8.39)	7.95 (7.64,8.27)	0.110
Plasma α,tocopherol mg/l (GM, 95% CI)	9.68 (9.54,9.82)	9.66 (9.35,9.98)	0.982
Vitamin C intake mg/d (GM, 95% CI)	119 (116-122)	123 (118-129)	0.109
Plasma ascorbate µmol/l (GM, 95% CI)	61.2 (59.8-62.8)	67.8 (65.1-70.6)	<0.001
Beta carotene intake mg/d (GM, 95% CI)	1.84 (1.77-1.90)	1.87 (1.76-1.99)	0.591
Plasma beta-carotene mg/l (GM, 95% CI)	0.254 (0.246-0.263)	0.296 (0.278-0.314)	<0.001
Child			
Girl n(%)	956 (49.7%)	241 (53.8%)	0.046
Birth weight g Median (IQR)	3460 (3090,3797)	3530 (3168,3830)	0.013

*GM = geometric mean

**p value: responders vs non responders, χ^2, Mann-Whitney, t-tests.

Table E2: Associations between total maternal vitamin D intake during pregnancy and eczema and hayfever outcomes in ten year old children.

	Quintiles of energy adjusted maternal vitamin D intake. Adjusted odds ratio (95% CI)*					p trend
	Q1 Low	Q2	Q3	Q4	Q5 High	
Postal questionnaire (n=934)						
Ever eczema	1	0.78 (0.49-1.25)	0.82 (0.52-1.31)	1.08 (0.69-1.68)	1.01 (0.65-1.57)	0.466
Doctor confirmed eczema	1	0.64 (0.39-1.04)	0.76 (0.47-1.23)	1.00 (0.63-1.58)	0.81 (0.51-1.30)	0.904
Medication to treat eczema in last year	1	1.00 (0.42-2.40)	1.54 (0.69-2.44)	1.91 (0.88-4.16)	1.20 (0.52-2.77)	0.278
Ever hayfever	1	1.11 (0.69-1.78)	1.00 (0.62-1.62)	0.65 (0.40-1.07)	0.99 (0.62-1.58)	0.336
Doctor confirmed hayfever	1	0.94 (0.51-1.75)	0.86 (0.46-1.60)	0.71 (0.38-1.34)	0.96 (0.53-1.75)	0.629
Medication to treat hayfever in last year	1	1.09 (0.65-1.82)	0.91 (0.54-1.53)	0.65 (0.37-1.12)	1.20 (0.73-2.00)	0.993
Longitudinal (1,2,5,10 year data)						
Eczema+	1	1.01 (0.80-1.28)	0.94 (0.74-1.19)	0.97 (0.76-1.24)	1.01 (0.79-1.28)	0.922
Hayfever+	1	1.06 (0.82-1.36)	0.90 (0.70-1.17)	0.91 (0.70-1.18)	0.97 (0.75-1.26)	0.481

*adjusted for maternal smoking during pregnancy, maternal atopy, birth order, child's sex, maternal age at recruitment, Scottish Index of Multiple Deprivation, birth weight, birth crown-heel length, birth head circumference, maternal vitamin E intake.
**Generalised estimating equations
+Discrete hazards modelling, hazard ratio

Tables E3: Associations between (a) total maternal vitamin E intake and (b) plasma α-tocopherol during pregnancy and eczema and hayfever outcomes in ten year old children.

(a)	Quintiles of energy adjusted maternal vitamin E intake. Adjusted odds ratio (95% CI)*					
	Q1 Low	Q2	Q3	Q4	Q5 High	p trend
Postal questionnaire (n=934)						
Ever eczema	1	0.74 (0.47-1.17)	1.26 (0.81-1.98)	0.99 (0.63-1.56)	0.96 (0.62-1.50)	0.674
Doctor confirmed eczema	1	0.80 (0.49-1.30)	1.15 (0.72-1.84)	1.01 (0.63-1.63)	0.87 (0.54-1.40)	0.93
Medication to treat eczema in last year	1	0.25 (0.09-0.69)	1.36 (0.68-2.71)	0.85 (0.40-1.79)	0.73 (0.34-1.56)	0.809
Ever hayfever	1	1.08 (0.67-1.74)	1.05 (0.65-1.70)	1.08 (0.67-1.75)	1.19 (0.74-1.90)	0.510
Doctor confirmed hayfever	1	0.97 (0.51-1.85)	1.01 (0.53-1.94)	1.22 (0.65-2.29)	1.21 (0.65-2.26)	0.371
Medication to treat hayfever in last year	1	1.10 (0.59-1.71)	1.08 (0.64-1.83)	0.99 (0.58-1.69)	1.11 (0.66-1.87)	0.724
Longitudinal (1,2,5,10 year data)						
Eczema+	1	0.73 (0.48-1.10)	1.04 (0.82-1.32)	0.91 (0.71-1.16)	0.95 (0.75-1.21)	0.579
Hayfever+	1	0.98 (0.76-1.28)	0.82 (0.62-1.07)	1.03 (0.80-1.33)	0.93 (0.72-1.21)	0.763

*adjusted for maternal smoking during pregnancy, maternal atopy, birth order, child's sex, maternal age at recruitment, Scottish Index of Multiple Deprivation, birth weight, birth crown-heel length, birth head circumference, maternal vitamin D intake.
**Generalised estimating equations
+Discrete hazards modelling, hazard ratio.

Tables E3: Associations between (a) total maternal vitamin E intake and (b) plasma α-tocopherol during pregnancy and eczema and hayfever outcomes in ten year old children.

(b)	Maternal α-tocopherol (μg/ml)	
	OR*$ (95% CI	p
Postal questionnaire (n=934)		
Ever eczema	0.71 (0.43-1.19)	0.198
Doctor confirmed eczema	0.74 (0.39-1.41)	0.365
Medication to treat eczema in last year	1.10 (0.98-1.24)	0.122
Ever hayfever	0.80 (0.37-1.71)	0.563
Doctor confirmed hayfever	0.58 (0.31-1.10)	0.096
Medication to treat hayfever in last year	1.04 (0.94-1.16)	0.429
Longitudinal (1,2,5,10 year data)		
Eczema+	1.03 (0.81-1.33)	0.800
Hayfever+	0.96 (0.73-1.26)	0.960

*adjusted for maternal smoking during pregnancy, maternal atopy, birth order, child's sex, maternal age at recruitment, Scottish Index of Multiple Deprivation, birth weight, birth crown-heel length, birth head circumference, maternal vitamin D intake.
$ OR expressed per SD increase in α-tocopherol, adjusted for plasma cholesterol

**Generalised estimating equations
+Discrete hazards modelling, hazard ratio.

Table E4: Associations between the vitamin D intake of children at age ten and wheeze and asthma outcomes at age ten.

	Quintiles of energy adjusted children's vitamin D intake. Adjusted odds ratio (95% CI)*					p trend
	Q1 Low	Q2	Q3	Q4	Q5 High	
Ever wheezed	1	0.96 (0.45-1.54)	0.52 (0.26-1.02)	0.77 (0.41-1.43)	1.02 (0.56-1.85)	0.962
Wheeze in last year	1	0.80 (0.36-1.77)	0.46 (0.19-1.16)	0.42 (0.32-1.65)	1.23 (0.58-2.58)	0.630
Wheezed in absence of a cold in last year	1	0.65 (0.26-1.66)	0.47 (0.17-1.31)	0.63 (0.24-1.63)	1.15 (0.50-2.64)	0.731
Doctor confirmed asthma	1	0.69 (0.33-1.42)	0.63 (0.30-1.32)	0.57 (0.27-1.23)	1.06 (0.54-2.08)	0.999
Asthma and wheeze in last year	1	1.20 (0.47-3.04)	0.60 (0.20-1.77)	0.84 (0.31-2.29)	1.65 (0.68-3.98)	0.424

*adjusted for maternal smoking during pregnancy, household smokers at age ten, maternal atopy, birth order, child's sex, maternal age at recruitment, Scottish Index of Multiple Deprivation, birth weight, birth crown-heel length, birth head circumference, children's vitamin E intake.

Table E5: Associations between the vitamin E intake of children at age ten and wheeze and asthma outcomes at age ten.

	Quintiles of energy adjusted children's vitamin E intake. Adjusted odds ratio (95% CI)*					P trend
	Q1 Low	Q2	Q3	Q4	Q5 High	
Ever wheezed	1	0.62 (0.33-1.16)	0.83 (0.45-1.52)	0.54 (0.28-1.03)	0.88 (0.49-1.60)	0.590
Wheeze in last year	1	0.67 (0.30-1.49)	0.31 (0.12-0.83)	0.52 (0.27-1.39)	1.20 (0.59-2.43)	0.630
Wheezed in absence of a cold in last year	1	0.70 (0.29-1.66)	0.27 (0.09-0.87)	0.48 (0.18-1.26)	1.00 (0.44-2.23)	0.786
Doctor confirmed asthma	1	1.30 (0.64-2.62)	0.62 (0.28-1.41)	0.76 (0.35-1.66)	1.51 (0.76-3.01)	0.602
Asthma and wheeze in last year	1	1.23 (0.50-3.07)	0.40 (0.12-1.32)	0.85 (0.32-2.28)	1.81 (0.78-4.22)	0.267

*adjusted for maternal smoking during pregnancy, household smokers at age ten, maternal atopy, birth order, child's sex, maternal age at recruitment, Scottish Index of Multiple Deprivation, birth weight, birth crown-heel length, birth head circumference, children's vitamin D intake.

Figure E1: Maternal vitamin D and E intakes dichotomised about the median, proportions of children with parental report of doctor confirmed asthma in the first ten years.