**ON-LINE SUPPLEMENT**

**Title: Multifaceted allergen avoidance during infancy reduces asthma during childhood with the effect persisting until age 18 years**

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## METHODS

**Subjects:** The study was approved by the Local Research Ethics Committee and written informed consent was obtained from the parents at recruitment and each follow-up. From February 1990 to February 1991, one hundred and twenty infants at high risk of developing atopy were recruited antenatally and randomised (using random allocation numbers) into prophylactic (n=58) and control (n=62) groups. The criteria for 'high risk' were: two or more members of the immediate family affected with an allergic disorder (asthma, atopic eczema or allergic rhinitis) or either parent or sibling affected with an allergic disorder plus cord serum IgE > 0.5ku/l in the infant. At recruitment parents completed a questionnaire seeking information on family history of allergy, household pets and smoking habits.

**Preventive measures during infancy:** A programme of reduced allergen exposure (food and aeroallergen) was instituted from birth for the infants in the intervention group. Lactating mothers followed a strict dietary regimen excluding dairy products, eggs, fish, and nuts up to 9 months or duration of the breast feeding, if shorter. Extensively hydrolysed hypoallergenic formula was given as a supplement, when needed. Dairy products, egg, wheat, nuts, fish and soya were excluded from the infants' diet for the first 9 months of life. These foods were gradually introduced from 9 months onwards. A dietician explained the dietary restrictions to mothers and written information was provided. The dietician regularly assessed the nutritional

adequacy of the mothers' and child's diet. Close supervision of mothers and child's weight and growth aspects was maintained throughout by the dietician and study nurses. Calcium and vitamin supplements were provided to all mothers.

Compliance with maternal and child's diet was excellent. We assessed this by analysis of random samples of breast milk for cows' milk proteins (β-lactoglobulin and casein). Eleven mothers had violated the dietary protocol. Eight mothers gave up the diet (self reported) at varying periods between 24 and 32 weeks and 3 mothers were found to have cows' milk protein in the breast milk and admitted to having cow's milk intermittently. All these participants were included in the analysis at all follow-ups.

Cot mattresses were covered with a polyvinyl impermeable cover. The carpets and upholstery in the infant's bedroom and lounge were repeatedly treated with an Acaracide (Acarosan, Crawford Chemicals, UK), from just before birth and then at 3 monthly intervals to the age of 9 months. This resulted in a five-fold reduction (from 25.9 μg/g dust at birth to 6.0 μg/g dust at 9 months) in dust mite antigen in the homes of the prophylactic group, while no significant change was observed in the control group.

**Control group:** Infants in the control group followed national guidelines recommended at that time, which advised breast feeding up to 6 months and introduction of solids after 4 months. They were also asked to avoid child's exposure to environmental tobacco smoke. However, compliance with these recommendations was not checked in the control group.

**STATISTICAL METHODOLOGY**

An intention to treat analysis was performed including all available data in the analysis. The sample size was limited to the original 120 participants who were recruited prenatally 18 years ago. For the 18-year follow-up, the primary outcome of this analysis was current asthma. Data were double entered and analysed using

SPSS version 19 (IBM, NY, USA) cross-sectionally (as at the 18-year follow-up) and then longitudinally, to include all follow-ups from 1 to 18 years of age (1, 2, 4, 8 and 18 years ). FeNO was not normally distributed and was log-10 transformed; post analysis it was back transformed for the purpose of reporting. The DRS was non-normally distributed and was log-10 transformed.

Bivariate analysis tested for differences in the characteristics of the two groups. For categorical variables, Pearson's chi-squared tests were used to test for significant differences (Fisher's exact test used when required by low expected cell counts). For continuous variables (age and household income), independent sample t-tests were used.

A binary logistic regression model (forward selection) was used to estimate the extent to which these differences affected the relative risk of having asthma at age 18. Six explanatory variables were tested for inclusion in the binary regression model: (1) group, (2) dual heredity, (3) family history of asthma (at least one parent or sibling with asthma) (4) whether or not the subject was a firstborn child, (4) exposure to smoke in the two years preceding the 18-year follow-up, and (6) maternal smoking during pregnancy. These six explanatory variables were added to the model one at a time, and likelihood ratio tests (LRTs) were used to compare goodness-of-fit of nested pairs of models. One of a nested pair of models was assumed to be a significantly better fit if the p-value of the LRT was less than 0.05. We tested for interaction between group and family history of asthma, but found this not to be a significantly better fit than the model containing the main effects for group and family history of asthma (p= 0.48).

To assess the relationship between group and the different types of asthma (see 'definitions' section), a multinomial logistic regression model was built using the same model-building strategy as for the binary regression model. The outcome variable had four categories: never had asthma, persistent asthma, remitted asthma

and late-onset asthma. The final multinomial logistic regression model contained the same covariates as the binary logistic regression model.

A second binary logistic regression model was built in exactly the same way in order to estimate the extent to which the relationship between group and atopy at age 18 was affected by the differences between the prevention and control groups. This model was based on the 103 individuals who underwent SPT and spirometry at age 18. None of the explanatory variables had a significant association with atopy at age 18 once the other variables were held constant, so the results presented in this paper for atopy are based on the bivariate analysis only.

Longitudinal analysis was undertaken using generalised estimating equations (GEEs) with a logit link function and an independent correlation structure. The GEE analysis was based on the 114 subjects who were followed up at 18 years as well as at age 1, 2, 4, and 8 (a total of 547 data points).

**ADDITIONAL RESULTS**

**Follow-up:** A high follow-up (114 of 120) was achieved, although 6 subjects were lost to follow-up. The reasons for this attrition are provided in the consort diagram. There was no significant difference between those seen and not seen at 18 year follow-up in key variables (Table E1).

**Table E1: Comparison of those seen and not seen at 17 years in key variables.**

| | Not seen at 18 yrs. (n=6) N (%) | Seen at 18 yrs. (n=114) N (%) | *P= |
|---|---|---|---|
| **Gender** | 1 (16.7) | 60 (52.6) | 0.1 |
| **Dual Heredity** | 5 (83.3) | 88 (77.2) | 1.0 |
| **Maternal smoking at birth** | 1 (16.7) | 38 (33.3) | 0.6 |
| **Cat at birth** | 3 (50.0) | 50 (43.9) | 1.0 |
| **Dog at birth** | 3 (50.0) | 49 (43.0) | 1.0 |
| **Wheeze at 8 yrs** | 2 (33.3) | 23 (20.2) | 0.6 |
| **Asthma at 8 yrs** | 2 (40.0) | 12 (11.4) | 0.1 |
| **Eczema at 8 yrs.** | 1 (16.7) | 38 (33.3) | 0.6 |

| | | | |
|---|---|---|---|
| **Rhinitis at 8 yrs.** | 1 (16.7) | 44 (38.6) | 0.4 |
| **Atopy at 8 yrs** | 1 (20.0) | 39 (34.8) | 0.7 |

Asthma: n=110; Atopy: n=117.

There were some differences between the prevention and control groups (Table E2): members of the prevention group were less likely to be first-born children (p=0.03), more likely to have dual heredity (p=0.01) and more likely to have a parent or sibling with asthma (p=0.01). The prevention group was less likely to have been exposed to tobacco smoke.

**Table E2. Demographic characteristics of participants at 18-year follow-up**.

| | Prevention (n=56) | Control (n=58) | p-value* |
|---|---|---|---|
| **Family history of asthma (at least one parent or sibling with asthma)** | 39 (69.6%) | 26 (44.8%) | 0.01 |
| **Dual heredity** | 49 (87.5%) | 39 (67.2%) | 0.01 |
| **First born child** | 14 (25.0%) | 26 (44.8%) | 0.03 |
| **Maternal smoking during pregnancy** | 7 (12.5%) | 15 (26.3%) | 0.06 |
| **Exposure to smoking in the home in the last 2 years** | 17 (30.4%) | 26 (44.8%) | 0.11 |
| **Current smoker** | 23 (41.1%) | 19 (32.8%) | 0.36 |
| **Either parent smoked at 1 year** | 20 (35.7%) | 25 (43.1%) | 0.42 |
| **Maternal allergy** | 41 (73.2%) | 39 (67.2%) | 0.49 |
| **Paternal allergy** | 31 (55.4%) | 32 (55.2%) | 0.98 |
| **Sibling allergy** | 34 (60.7%) | 28 (48.3%) | 0.18 |
| **Male** | 27 (48.2%) | 34 (58.6%) | 0.27 |
| **Living with parents** | 48 (85.7%) | 53 (91.4%) | 0.34 |
| **In full time education** | 37 (66.1%) | 42 (72.4%) | 0.46 |
| **Total cord IgE >0.5 kU/l** | 14 (35.9%) | 18 (39.1%) | 0.76 |
| **Cat at home in the last 2 years** | 20 (35.7%) | 21 (36.2%) | 0.96 |
| **Mean age in years (standard deviation)** | 18.4 (0.4) | 18.5 (0.4) | 0.24 |
| **Mean annual family income (standard deviation)** | £26,660 (£14,455) | £25,510 (£12,937) | 0.66 |

* p-values are from chi-squared tests, except for the continuous variables age and family income, for which p-values are from independent samples t-tests.

**Asthma Definition:** Our asthma definition included three components, physician diagnosed asthma, current wheeze or current treatment. When these components were analysed separately in a univariate analysis, the differences did not reach statistical significance. However, “ever asthma treatment” was significantly different between the groups (Table E3).

**Table E3: Individual components of asthma definition in prevention and control groups.**

| | Prevention (n=56) | Control (n=58) | OR (95%CI) | *P= |
|---|---|---|---|---|
| **Physician diagnosed asthma** | 16 (28.6%) | 26 (44.8%) | 0.49 (0.23-1.07) | 0.08 |
| **Current wheeze** | 7 (12.5%) | 15 (25.9%) | 0.41 (0.15-1.1) | 0.09 |
| **Current asthma treatment** | 5 (8.9%) | 11 (19.4%) | 0.42 (0.14-1.29) | 0.18 |
| **Ever asthma treatment** | 10 (17.9%) | 22 (37.9%) | 0.36 (0.15-0.85) | 0.02 |

**Objective markers of asthma:** We have analysed objective markers for asthma for all participating children, where these data were available. There were no statistically significant differences, although eosinophils showed a trend towards higher values in the control group (Table E4).

**Table E4: Objective markers of lung function, airway responsiveness and inflammation in all participants.**

| | N= | Prevention | N= | Control | *P= |
|---|---|---|---|---|---|
| **FEV1 (% predicted)** | 54 | 96.8 (13.0) | 48 | 98.6 (10.9) | 0.5 |
| **FVC (%predicted)** | 54 | 95.2 (12.5) | 48 | 97.9 (12.6) | 0.3 |
| **FEV1/FVC** | 54 | 102.6 (9.7) | 48 | 100.9 (8.0) | 0.4 |
| **FEF25-75 (% predicted)** | 51 | 97.4 (24.1) | 48 | 94.9 (18.1) | 0.6 |
| **Peak Exp Flow** | 51 | 96.0 (19.3) | 48 | 98.0 (14.9) | 0.5 |
| **DRS (log10)** | 47 | 0.82 (1.1) | 43 | 0.80 (1.0) | 0.9 |
| **FeNO** | 49 | 20.89 (1.5) | 54 | 25.12 (2.1) | 0.16 |
| **Eosinophils (%)** | 19 | 0.5 (0.0-2.5) | 24 | 0.8 (0.3-4.9) | 0.08 |
| **Neutrophils (%)** | 19 | 9.5 (2.8-16.8) | 24 | 5.7 (1.4-16.1) | 0.4 |
| **Epithelial cells (%)** | 19 | 2.8 (1.0-9.0) | 24 | 5.3 (2.9-10.6) | 0.5 |

DRS = dose response slope, FeNO = exhaled nitric oxide. Numbers are means (standard deviation) or median (interquartile range; 25-75) except for FeNO which is geometric mean and SD. P-values are two-sample T-test (means) except for induced sputum results (Mann Whitney U Test).

**Allergic sensitisation:** There were 12 children in the prevention group and 6 in the control group who had developed allergic sensitisation (atopy) between 8 and 18 years. This was primarily driven by grass pollen, house dust mite and cat allergens sensitisation but the pattern was similar in the two groups with no statistically significant differences. Most of these children (10 of 18) developed allergic rhinitis. Only one subject had new onset asthma and no one had new-onset eczema.

**Table E5: New onset of sensitisation to common allergens in the prevention and control groups.**

| | Prevention (n=12) N (%) | Control (n=6) N (%) | P= |
|---|---|---|---|
| **Clinical Allergic Conditions** | | | |
| **Asthma** | 1 (8.3) | 0 | 1.00 |
| **Eczema** | 0 | 0 | _ |
| **Rhinitis** | 7 (58.3) | 3 (50.0) | 1.00 |
| **Allergic sensitisation** | | | |
| **House dust mite** | 6 (50.0) | 4 (66.7) | 0.63 |
| **Grass pollen** | 8 (66.7) | 1 (16.7) | 0.13 |
| **Tree pollen** | 3 (25.0) | 0 | 0.52 |
| **Cat** | 3 (25.0) | 1 (16.7) | 1.03 |
| **Dog** | 5 (41.7) | 0 | 0.11 |
| **Cladosporium** | 1 (8.3) | 0 | 1.00 |
| **Alternaria** | 2 (16.7) | 1 (16.7) | 1.00 |
| **Cow's milk** | 2 (16.7) | 0 | 0.53 |
| **Wheat** | 6 (50%) | 1 (16.7) | 0.32 |
| **Peaanut** | 2 (16.7) | 0 | 0.53 |
| **Soya** | 1 (8.3) | 0 | 1.00 |