PROTECTIVE FACTORS IN CHILDHOOD ALLERGY RELATED TO DIET AND LIFESTYLE

Helen Rosenlund
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ABSTRACT

The prevalence of allergic disease among children has increased markedly during several decades. The reasons behind the increase remain unclear, but lifestyle and environmental factors associated with the Western way of living seem to be of importance. The Western lifestyle includes changes in diet, reduced infant infections, increased use of antibiotics, antipyretics and vaccinations, all of which have been investigated in relation to allergic disease, but the role of these factors for development of allergic disease has not been clarified.

The aim of this thesis was to investigate if certain dietary and lifestyle factors could have a protective effect on the development of allergic disease in childhood. It is based on two epidemiological materials, the multinational PARSIFAL study (including Steiner school children, farm children and two reference groups in five European countries), and the Swedish BAMSE birth cohort study. PARSIFAL had a cross-sectional design with nearly 15000 children aged 5-13 years. BAMSE included more than 4000 children, who were followed from birth until 8 years of age.

Focusing on the 4600 Steiner school children and their 2000 referents in the PARSIFAL study, we could in a European context confirm the lower prevalence of allergic disease among Steiner school children observed in a previous Swedish study. Use of antibiotics and antipyretics during the first year of life was associated with increased risks of asthma and eczema, while an increased risk of rhinoconjunctivitis was related to antibiotic use. However, we can not exclude that other factors in the anthroposophic lifestyle contributed to these results.

We also studied the association between measles vaccination and measles infection and allergic disease among all children in the PARSIFAL study. To reduce disease-related modification of exposure we excluded children with early symptoms of allergic disease in some analyses. After this exclusion, no association was observed between measles vaccination and allergic disease, while measles infection was inversely related to allergic disease (OR 0.65, 95% CI 0.45-0.95).

In the BAMSE cohort, 2400 children provided information on diet and allergic disease at 8 years of age. A high intake of fruits was inversely associated with allergic rhinitis, but after considering disease-related modification of consumption, that is, excluding all children who reported food related allergic symptoms, most associations became non-significant. Furthermore, when we investigated the role of multivitamin supplementation no association was observed between current use and risk of allergic disease, while an inverse association was indicated between supplementation with multivitamins in early life and sensitization to food allergens (OR 0.61, 95% CI 0.39-0.97).

It may be concluded that disease-related modification of exposure (reverse causation) is an important aspect to consider in studies on behavioral factors and allergic disease. This is especially important in cross-sectional studies, but the fact that the natural history of allergic disease might cause the same child to experience different symptoms at different ages, suggests that disease-related modification of exposure may be a possible confounding factor also in prospective studies.

Key words: allergy, antibiotics, antipyretics, asthma, biodynamic, diet, eczema, fruits, measles infection, measles vaccination, multivitamins, rhinitis, sensitization, vegetables
LIST OF PUBLICATIONS

The thesis is based on the following papers and manuscripts which will be referred to in the text by their Roman numerals.

   Allergic disease and atopic sensitization in Steiner school children.

II. **Helen Rosenlund**, Anna Bergström, Johan S Alm, Jackie Swartz, Annika Scheynius, Marianne van Hage, Kari Johansen, Bert Brunekreef, Erika von Mutius, Markus J Ege, Josef Riedler, Charlotte Braun-Fahrländer, Marco Waser, Göran Pershagen and the PARSIFAL study group.
   Measles vaccination and measles infection in children in relation to allergic disease and atopic sensitization.

III. **Helen Rosenlund**, Inger Kull, Alicja Wolk, Göran Pershagen, Magnus Wickman, Anna Bergström.
   Fruit and vegetable consumption in relation to allergy – disease related modification of consumption?
   *Submitted* 2010

IV. Kristin Marmsjö, **Helen Rosenlund**, Inger Kull, Niclas Håkansson, Magnus Wickman, Göran Pershagen, Anna Bergström.
   Use of multivitamin supplements in relation to allergic disease in 8 year old children.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAMSE</td>
<td>Swedish abbreviation for: children, allergic disease, milieu, Stockholm, an epidemiologic study.</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food frequency questionnaire</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>ISAAC</td>
<td>International study of asthma and allergy in childhood</td>
</tr>
<tr>
<td>LRI</td>
<td>Lower respiratory tract infection</td>
</tr>
<tr>
<td>MMR</td>
<td>Measles, mumps, rubella</td>
</tr>
<tr>
<td>OAS</td>
<td>Oral allergy syndrome</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PARSIFAL</td>
<td>Prevention of allergy - risk factors for sensitization in children related to farming and anthroposophic lifestyle</td>
</tr>
<tr>
<td>Th1/2</td>
<td>T helper 1 or 2</td>
</tr>
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</table>
1 INTRODUCTION

1.1 EPIDEMIOLOGY OF ALLERGIC DISEASE

The prevalence of IgE-mediated allergic disease has increased markedly in Western developed countries in the final decades of the twentieth century, particularly among children \(^1\). However, recent reports indicate that the occurrence has stabilized, while in less developed countries the prevalence is still increasing \(^2-4\). The reason for the increase is unclear, but lifestyle and environmental factors associated with the Western way of living seem to be of importance. The Western lifestyle includes changes in diet, reduced infant infections, increased use of antibiotics, antipyretics and vaccinations, all of which have been investigated in relation to allergic diseases, but none of these factors alone can explain the trends in prevalence of allergic disease \(^5, 6\). Genetic changes in populations would be too slow to account for such rapid changes in prevalence \(^7\). However, most investigators seem to agree that a complex interaction between genetic and environmental factors regulates the expression and development of allergic disease \(^8, 9\).

1.2 NOMENCLATURE

The nomenclature of allergic diseases has changed over the years. This has made it difficult to compare symptoms and diagnoses in research as well as in the clinic. Hypersensitivity is often classified by its immunologic component, and may be divided in IgE-mediated or non-IgE-mediated disease. A schematic overview of the nomenclature of the allergic disorders, as proposed by Johansson and colleagues in 2004 \(^10\) is presented in Figure 1.

![Figure 1. The nomenclature for allergic diseases proposed 2004 by Johansson and colleagues.](attachment:\figure1.png)
The term hypersensitivity describes objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons. Atopy is a personal or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens \(^{10}\). As a consequence, these persons can develop typical symptoms of allergic diseases that are primarily manifested by rhinitis, asthma and eczema. Asthma is characterized by a chronic inflammation in the airways. Symptoms include airway obstruction and bronchial hyperreactivity, which often lead to recurrent episodes of wheezing, breathlessness and coughing \(^{11}\). Allergic rhinitis is characterized by an itchy and runny nose, accompanied by sneezing and often red itchy eyes \(^{11}\). Eczema is characterized by hypersensitivity reactions in the skin on certain locations, resulting in red itchy rashes \(^{11}\).

The “allergic march” refers to the natural history of allergic disease, and to the typical time sequence of the symptoms that appear during a certain period in life, persists over a few years, and often show a tendency for spontaneous remission \(^{9}\). Eczema is most prevalent in infancy, followed by wheeze and asthma that is more frequently diagnosed in young children before school start. Rhinitis is most prevalent in school children \(^{12-14}\).

### 1.3 RISK AND PROTECTIVE FACTORS FOR ALLERGIC DISEASE

Several risk factors for childhood allergy have been investigated during the past decades, for example, parental smoking, air pollution, heredity, breastfeeding, number of siblings, socio-economic status, obesity, and household pets. Parental smoking and heredity are probably the most well-established risk factors for childhood allergic disease. Parental smoking, especially during pregnancy or the child’s first year of life, has consistently been associated with increased risks for wheezing and asthma in childhood \(^{6,7}\). Heredity (also known as family history or parental allergic disease) is a major risk factor for allergic disease particularly in childhood \(^{15}\), while the associations between the other risk factors and allergic disease are inconsistent \(^{6,16,17}\).

The hygiene hypothesis is the most well-known theory about the increase in allergic disease today \(^{18}\). It postulates that changes in exposure to infectious diseases and microbial products associated with a cleaner Western lifestyle have resulted in an imbalance in the developing immune system \(^{18}\). As a consequence, there are no longer sufficient amounts of microbial factors that may have an allergy-preventing potential. Normally there is a balance in the immune response between Th1 (T-helper 1) and Th2 cells, but in allergic individuals a domination of Th2 cells is present, accompanied by a cytokine profile that promotes allergic disease. The reduced activity of regulatory T cells may also play a part in this by suppressing the immune system \(^{19-21}\).

#### 1.3.1 Diet

The fact that what we eat might affect the development of allergic disease has been confirmed in several studies \(^{22-24}\). Modern diets differ in many aspects from more traditional diets, with more complex, processed and synthetic foods and less fresh fish, fruits and vegetables \(^{25}\). Certain components of a diet, for example fatty acids or antioxidants, have been observed to have anti-inflammatory effects \(^{25,26}\). The Western diet includes more of linoleic acid, with the end-metabolite arachidonic acid, which
may increase the production of inflammatory mediators. Antioxidants could decrease oxidative processes because of their ability to scavenge free radicals, for example in the lung epithelium of asthmatics. Another theory is based on the fact that the consumption of fruit and vegetables has been decreasing during the same time period as the prevalence of allergic disease has increased, which may imply a casual relationship.

The literature on the relation between different types of diets and allergic disease is sparse. An organic diet has been related to allergic disease in children and the results suggest that organic dairy products could have a protective effect on eczema, while other organically produced foods were not associated with the development of allergic disease. The number of studies on the relation between different types of diets and allergic disease in childhood is very limited.

Allergic disease has also been associated with consumption of specific foods. Intake of fruit and vegetables has mostly been associated with lowered risks of allergic disease. Many children who are sensitized against tree or grass pollen cross-react with certain fruits and vegetables, which makes the consumption of fruits and vegetables highly affected by the presence of allergic disease. This implies that people with allergic diseases avoid or exclude certain fruits or vegetables from their diet. Such aspects have generally not been taken into account in studies on the relation between fruit and vegetable consumption and allergic disease.

The consumption of dietary supplements among children has become quite common. A Swedish national survey performed in 2003 showed that 33% of the children used vitamin supplements, mostly multivitamins. However, the association between multivitamin supplementation in children and development of allergic disease has been studied to a limited extent, and results are inconsistent.

1.3.2 Lifestyle

Several studies have reported that children raised in farming environments have a lower prevalence of allergic disease and atopic sensitization compared to other children. The protective effect has been associated with exposure to dust (endotoxin), consumption of farm milk, contact with farm animals, and the gut microflora.

The anthroposophic lifestyle has been less studied in relation to allergic disease. Anthroposophy is a spiritual philosophy founded in the early 20th century by Rudolf Steiner and it has been applied to education, medicine, art, architecture, and agriculture (biodynamic farming). Anthroposophic children often receive less antibiotics and antipyretics, have a high consumption of organic and biodynamic foods, and receive fewer vaccinations which are usually given later in life. Only one previous study has investigated the association between lifestyle exposures and allergic disease in anthroposophic children, who often attend Steiner schools. However, in this study it was not possible to identify a single lifestyle factor that was primarily responsible for the lower prevalence of atopy in these children, because the exposures were strongly correlated. To disentangle the relation between different lifestyle factors and allergic disease among Steiner school children, further studies are needed in a more heterogeneous group of Steiner school children.
Infection with measles virus has a strong impact on the immune system and might affect the development of allergy \(^{44}\). Studies on the effect by measles infection on allergic disease have shown conflicting results \(^{45-47}\). Measles vaccination has also been associated with the development of allergic disease, but the evidence is inconsistent \(^{46,48-50}\). The results from studies that considered that the allergic disease could have affected these exposures also appear inconsistent \(^{51-54}\).
2 AIMS

The main purpose of this thesis was to investigate if certain dietary and lifestyle factors could have a protective effect on the development of allergic disease in childhood.

The specific aims were:

- To identify possible protective factors for allergic disease related to the anthroposophic lifestyle.
- To study the association between measles vaccination and measles infection and allergic disease.
- To investigate the association between fruit and vegetable consumption and allergic disease.
- To explore the association between multivitamin supplementation and allergic disease.
3 MATERIAL AND METHODS

The papers in this thesis are based on two epidemiologic materials; the cross-sectional PARISFAL study, and the BAMSE birth cohort study. Paper I and II are based on the PARISFAL study and paper III and IV on the BAMSE study.

3.1 THE PARSIFAL STUDY

3.1.1 Study design and study population

PARSIFAL (Prevention of allergy – Risk factors for sensitization in children related to farming and anthroposophic lifestyle) is a cross-sectional multicentre study conducted in Austria, Germany, the Netherlands, Sweden and Switzerland. The study included 5-13 year old children who represented two different populations; Steiner school children and farm children, as well as two reference groups. The overall aim of the study was to assess the role of environmental and lifestyle factors for development of allergic disease in children, with a focus on protective factors. The study was approved by local ethics committees in all participating countries 55.

Steiner school children and Steiner reference children

In all countries, children were recruited from classes in Steiner schools in Sweden. Children in these schools often come from families with an anthroposophic lifestyle. All children in the selected Steiner schools were invited to participate. Corresponding reference children were selected from non-Steiner schools in the vicinity. In Germany and Switzerland it is quite common that “non-anthroposophic” parents send their children to Steiner schools, and therefore an oversampling of children was made. To be sure that German and Swiss Steiner school children had an anthroposophic lifestyle they were selected through specific questions in the questionnaire.

Farm children and farm reference children

Farm children were defined as children currently living on a farm and whose family runs the farm. In Germany, the Netherlands and Switzerland farm children were selected from schools in rural areas known to have a high percentage of farmers. In Austria the school teachers in rural areas selected children from farming families. The farm reference children went to schools in the same areas as the farm children, but did not live on a farm or have a family who run a farm. In Sweden, farmers with children were identified from the Farming Registry at the National Bureau of Statistics and farm reference children were randomly selected from the population registry among children living in the same area.

3.1.2 The questionnaire

Parents who consented to that their child took part in the study answered a detailed questionnaire which included questions on environmental exposures, lifestyle, socio-
economic conditions, history of infections and vaccinations, diet, contact with animals, and on symptoms and diagnoses of allergic disease. Most of the questions were based on the internationally validated and translated ISAAC (International study of asthma and allergy in childhood) phase-II questions, but also on a similar study in Austria, Germany and Switzerland [39], the BAMSE study [56] and a previous Swedish study on children with anthroposophic lifestyle [43]. In total, the questionnaire was distributed to 21905 children. The distribution and collection took place between October 2000 and May 2002, during largely overlapping time periods in the five countries.

3.1.3 Participation rate and inclusion in the study

Of the 21905 children invited to take part in the study 15137 children provided questionnaire data. The overall participation rate was 69% and differed between the countries, from about 80% in Austria and Switzerland, 75% in Germany, 71% in Sweden and 50% in the Netherlands. Participation differed less in the different child groups. A total of 244 children were excluded because they were outside the age limits (5-13 years old) or lacked information on sex or group belonging, leaving 14893 children (50% boys and 50% girls) for the analyses.

Of the 14893 children, 19% were farm children, 37% were farm reference children, 31% were Steiner school children, and 14% were Steiner reference children. Figure 2 presents the number of included children in each child group in the different countries.

![Figure 2. The total number of children in each child group from the different countries who were included the PARSIFAL study (N=14893).](image-url)
3.1.4 Clinical investigation and blood sampling

The clinical investigation was performed by specially trained nurses and included blood sampling, weight and height measurements, as well as collection of information about the child’s immunizations. In Austria, the Netherlands and Sweden all children whose parents had consented to blood sampling were invited to a clinical investigation. In Germany and Switzerland only a random sample of those who consented were invited, because of the comparatively large number of children included in these countries.

In all, parents of 8788 children gave consent for blood sampling and 4854 children were invited to the clinical examination. Of these, 4049 children provided a blood sample.

3.1.5 Definition of exposures

Specific questions from the questionnaire were used for analyzing different lifestyle exposures. Many of these exposures are associated with the anthroposophic lifestyle.

Antibiotic use was defined as a positive answer to the question “Has your child ever used antibiotics”. Parents of antibiotic users further specified the child’s age at first use in one of four pre-defined categories (0-6 months, 7-12 months, 13-24 month, >24 months).

Antipyretic use was defined as a positive answer to the question “Has your child ever used antipyretics”. Parents of antipyretic users further specified the child’s age at first use in one of four pre-defined categories (0-6 months, 7-12 months, 13-24 month, >24 months).

Measles vaccination was defined as a positive answer to the question “Has your child been vaccinated against measles?”

MMR vaccination was based on positive answers to all the following questions “Has your child been vaccinated against measles?”, “Has your child been vaccinated against mumps?”, “Has your child been vaccinated against rubella?”

Biodynamic diet was based on the following question “Has your child’s diet mainly been based on a) conventional food, b) organic food, c) biodynamic food?” If the parent only answered biodynamic food the child was defined as having a biodynamic diet. A conventional diet was defined accordingly, while “other” diets were defined if the diet was mainly based on organic food or combinations of conventional, organic and/or biodynamic foods.

3.1.6 Definition of health outcomes

In PARSIFAL, all health outcomes were reported by the parents, except atopic sensitization which was assessed from blood sampling.
Current rhinoconjunctivitis symptoms were defined as sneezing, runny nose, nasal block-up and itchy eyes in the child during the last 12 months without having a cold at the same time.

Doctor’s diagnosis of hay fever was defined as ever having received a doctor’s diagnose of rhinoconjunctivitis.

Current wheezing was defined as having wheezing at least once during the last 12 months.

Doctor’s diagnosis of asthma was present in children ever diagnosed with asthma, or obstructive bronchitis more than once.

Current atopic eczema symptoms were present if the child ever had had an itchy rash intermittently for at least 6 months, and if the child had had an itchy rash during the last 12 months.

Doctor’s diagnosis of atopic eczema was present in children with atopic eczema and who ever had had an itchy rash lasting at least 6 months.

Any allergic symptom was indicated if the child has had symptoms of allergic disease (that is, current rhinoconjunctivitis symptoms, current wheezing, and/or current atopic eczema symptoms), or any combination or these.

Any doctor’s diagnosis of allergy was indicated if the child had doctor’s diagnose of rhinoconjunctivitis, asthma, and/or atopic eczema, or any combination of these.

Atopic sensitization was indicated if the child had at least one allergen-specific serum IgE result of ≥0.35 kU A/L against common inhalant allergens (Phadiatop; birch, timothy, mugwort, Dermatophagoides pteronyssinus and farinae, cat- dog- and horse epithelium, and Cladosporium herbarum) and/or food allergens (fx5; egg white, milk, fish, wheat, peanut, and soy), ImmunoCAP System, Phadia AB, Uppsala, Sweden. In addition, a cut-off value of ≥3.5 kU A/L was used in some analyses in paper II. All IgE analyses were performed at the Department of Clinical Immunology, Karolinska University Hospital Solna, Stockholm, Sweden.

Measles infection was defined if the question “Has your child had measles?” was answered with a “yes”.
3.2 THE BAMSE STUDY

3.2.1 Study design and study population

This ongoing birth cohort started in 1994 and comprised 4089 infants from predefined areas of Stockholm, Sweden. The children were born between February 1994 and November 1996 and the parents were informed about the study at their first visit at the Child Health Centre when the child was about 2 months old. The main objective of the BAMSE (Swedish abbreviation for Children, Allergic disease, Milieu, Stockholm, an Epidemiologic study) study is to examine relationships between certain environmental factors and development of allergic disease in children. The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm, Sweden.

3.2.2 Questionnaires

The first questionnaire collected data on environmental exposures (smoking, housing conditions etc) and parental allergic disease and was handed out by the Child Health Centre nurse or sent by mail. After that, the families were contacted with a follow-up questionnaire when the children were 1, 2, 4 and 8 years old (figure 3). The questionnaires at follow-up collected data on symptoms related to allergic disease as well as key exposures, such as parental smoking.

3.2.3 Participation rate and inclusion in the study

A total of 7221 infants were born between February 1994 and November 1996 in the predefined areas of Stockholm. Of these, 477 families could never be reached. Moreover, families were excluded from the study if they were planning to move within the coming 12 months, did not have sufficient knowledge of Swedish, had an infant suffering from a severe disease, or already had an older child enrolled in the study (1256 children). In total, 5488 children were eligible for the study. Of these, 1399 families never answered or declined participation. The final cohort consisted of 4089 new-born infants (51% boys and 49% girls), representing 75% of all eligible infants during that period.

A short questionnaire about parental allergic disease and parental smoking was sent to 1418 of the non-responding or excluded families to find out if these groups differed from the participating families. No difference was observed regarding parental allergic disease, but in non-responding or excluded families parents smoked to a significantly higher extent compared to the parents in the participating families (maternal smoking 17% vs. 9%, paternal smoking 23% vs. 18%).
3.2.4 Clinical investigation and blood sampling

All children with completed questionnaires at age 8 years were invited to a clinical investigation including blood sampling. The clinical investigation took place at the Department of Occupational and Environmental Health, Stockholm, where height and weight measures as well as blood sampling was performed by trained nurses.

Sera of 2470 children (60%) were analyzed for specific IgE to common inhalant and food allergens.

3.2.5 Assessment of diet

At the clinical examination at the 8 year follow-up, parents (together with their child) were asked to answer a food-frequency questionnaire (FFQ) containing questions about 98 foods and beverages frequently consumed in Sweden. Food groups listed in the FFQ were pasta/cereals, potatoes, meat, fish, eggs, vegetables, fruits, snacks/candy/cookies, drinks and other. The FFQ contained questions about the frequency of consumption and the participants were asked how often, on average, over the past year they had consumed each item. Eight categories for frequency of consumption were provided, ranging from never to three times per day or more. Furthermore, the food questionnaire included a question about use of vitamin supplements.

In total, 2614 families (64%) filled out the FFQ. Most often the FFQ was filled out by a parent (57%) or by a parent together with their child (40%).

3.2.6 Definition of exposures

Consumption of fruit and vegetables

Information on fruit and vegetable consumption was collected from the FFQ completed at the 8 year follow-up. The FFQ contained questions about the frequency of...
consumption of 5 fruit items and 13 vegetable items. The frequency responses were converted into average daily consumption of each fruit and vegetable item. Thus, no information on the amounts of each serving was available.

“Total fruit consumption” included apples/pears (included in the FFQ as one food item), citrus fruits, bananas, berries, and other fruits. Fruit juice was not included in the group of total fruits. “Total vegetable consumption” included carrots, cabbage, cauliflower, broccoli/Brussels sprouts, turnips/beets, lettuce, tomato, cucumber, spinach/kale, onions/leek, green peas, pea soup (from dried peas) and beans (brown, white, soy)/lentils. We also combined specific vegetables into the following 2 subgroups: cruciferous and green vegetables (cabbage, cauliflower, broccoli/Brussels sprouts, turnips/beets, spinach/kale) and legumes (green peas, pea soup, beans/lentils).

**Vitamin supplementation**

Information about vitamin supplementation was also collected from the FFQ at the 8 year follow-up. The use of vitamin supplements during the last 12 months was reported to be either regular, occasional, or none (figure 4). Parents of regular and occasional users further specified what kind of predefined supplement their child had used (multivitamins with minerals; multivitamins without minerals; vitamins A and D (combined); vitamin A; vitamin D; vitamin C; other), how many pills they consumed per week, and when the supplementation started.

**Has your child used any vitamin supplement during the past 12 months?**
- □ Yes, regularly
- □ Yes, occasionally
- □ No

**If yes, which or what vitamin supplements have your child used?**

<table>
<thead>
<tr>
<th>Vitamin Supplementation</th>
<th>How many pills per week?</th>
<th>Since what year?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multivitamins with minerals</td>
<td>________________________</td>
<td></td>
</tr>
<tr>
<td>Multivitamins without minerals</td>
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<td>AD-vitamins</td>
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<td>Vitamin D</td>
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<tr>
<td>Vitamin C</td>
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<td></td>
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<tr>
<td>Other, what?</td>
<td>________________________</td>
<td></td>
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</tbody>
</table>

*Figure 4. Questions about current vitamin supplementation included in the FFQ at the 8-year follow-up in the BAMSE study.*
3.2.7 Definition of health outcomes

Definition of asthma, allergic rhinitis, and eczema, were based on the follow-up questionnaire at age 8 years.  

Allergic rhinitis was defined as showing symptoms of sneezing, a runny or blocked nose, or itchy, red and watery eyes after exposure to furred pets or pollen (according to the questionnaire), or having received a doctor’s diagnosis of allergic rhinitis between the time of the previous questionnaire at age 4 years and the questionnaire at age 8 years.

Asthma was defined as having at least 4 episodes of wheeze in the last 12 months or at least 1 episode of wheeze during the same period in combination with prescribed inhaled steroids occasionally or regularly.

Eczema was defined as dry skin in combination with itchy rash for at least 2 weeks with typical localization during the last 12 months, and/or a doctor’s diagnosis of eczema from the age of 4 years.

Any allergic disease was indicated if the child had asthma, allergic rhinitis or eczema at 8 years of age, or any combination of these diseases.

Atopic sensitization was indicated if the child had at least one allergen-specific IgE result of \( \geq 0.35 \) kU A/L against common inhalant allergens: cat, dog, horse, birch, timothy, mugwort, Dermatophagoides pteronyssinus, and Cladosporium species (Phadiatop) and/or food allergens: cows milk, hens egg, cod fish, soy bean, peanut and wheat (fx5), (ImmunoCAP, Phadia AB, Uppsala, Sweden).

3.3 STATISTICAL ANALYSES

Logistic regression was used to investigate the association between certain exposure factors and allergic disease and/or atopic sensitization. The results are presented as odds ratios (OR) with 95% confidence interval (95% CI).

A confounder analysis was made to evaluate possible confounding factors. If the OR changed with more than 10% when entering the potential confounding variable in the crude model the variable was included in the final model. In paper I and II “traditional risk factors” for allergic disease were also included as potential confounding factors. In paper III, potential risk factors related to fruit and vegetable consumption in our study were also included in the model. In paper IV, no additional confounders were chosen than those included through the confounder analysis.

Differences in the distribution of selected characteristics between groups were tested with the chi-square test. The Wald test was used to assess interaction between covariates (departure from a multiplicative model). To assess cross-country heterogeneity in paper I, separate estimates for each country and a pooled weighted estimate using random-effect meta-analysis were calculated. Statistical significance was calculated by the Pearson \( \chi^2 \) test statistic and defined as a P value <0.05. Different
analyses were made to reduce the effects of disease-related modification of exposure (reverse causation). In paper II and IV, children with early symptoms of wheezing and/or eczema were excluded from or adjusted for in the model. In paper III we excluded children who reported food related allergic symptoms (wheezing, itchy eyes and/or runny nose, oral allergy syndrome, eczema, urticaria, or diarrhea/vomiting) from specific fruits or vegetables (apple, peach, kiwi, avocado, banana, fresh carrot, or “other”). This was made to reduce any effect from children whose exposure to the studied factors (measles infection, measles vaccination, fruit and vegetable consumption, and vitamin supplementation) changed because of earlier symptoms of allergy.

All statistical analyses were performed with STATA Statistical Software (release 8.0; StataCorp, College Station, TX).
4 RESULTS

4.1 THE PARSIFAL STUDY

4.1.1 Prevalence of allergic disease

The prevalence of allergic disease and atopic sensitization differed between the different countries and child groups. Figure 5 presents the prevalence of allergic disease among farm children, Steiner school children and their reference groups. In total, farm children had a statistically significantly lower prevalence of all allergic diseases compared to the farm reference children, except for current atopic eczema symptoms. Similar results were found for Steiner school children compared to Steiner reference children, although not statistically significant for current wheezing, and doctor’s diagnose of atopic eczema.

![Prevalence of allergic disease and atopic sensitization among the different child groups in the PARSIFAL study (N=14893 children).](image)

In total, 27% of all children provided a blood sample and this number differed in the different countries; Austria 60%, Germany 16%, Sweden 75%, Switzerland 27%, and the Netherlands 17%. Overall, children who provided a blood sample had similar characteristics and prevalence of allergic disease as the other children in the respective group, except among Steiner reference children where children with blood samples had
a somewhat higher prevalence of allergic disease (36%) than those without (31%) 55. Differences in symptom/disease rates related to blood samples between Steiner and Steiner reference children tended to be most pronounced in Sweden, Switzerland, and the Netherlands. This dissimilarity was not observed among farm or farm reference children who provided a blood sample.

The lower prevalence of allergic disease and atopic sensitization observed among farm and Steiner school children compared to their reference group, was also seen in country-specific analyses. Adjustments for prevalence of any allergic disease or symptom had no major effect on the country specific results regarding atopic sensitization. Farm children had a lower prevalence of allergic disease, compared to farm reference children, in all countries.

4.1.2 Lifestyle factors

Children who were included in the analyses of paper I were Steiner school children and Steiner reference children who had completed the questionnaire. In total, 6630 children were included of which 4606 were Steiner school children and 2024 Steiner reference children.

Considerable difference was observed when exposures related to anthroposophic lifestyle were studied among Steiner school children and their referents. Nearly 42% of the Steiner school children had never used antibiotics, but only 15% among Steiner reference children. A similar finding was noted for antipyretic use, 43% and 8%, respectively. Furthermore, 15% among Steiner school children consumed a diet based on biodynamic food products and only 1% in the Steiner reference group. MMR vaccination was about 3 times more common in the reference group, and consequently, the prevalence of measles infection was 33% among Steiner school children compared with 10% among the Steiner reference children.

The associations between doctor’s diagnosed allergic disease and specific anthroposophic lifestyle factors are presented in figure 6. Use of antibiotics during first year of life was positively associated with all studied outcomes, except atopic sensitization. Antipyretic use was positively associated with doctor’s diagnosed asthma and current atopic eczema symptoms, while no significant associations were observed for a biodynamic diet and allergic disease.
In the analyses of the relation between antibiotic use and allergic disease we made an attempt to reduce the potential effect of reverse causation, that is, children with allergic symptoms (especially wheeze and asthma) could receive antibiotics on the presumption that they had a bacterial respiratory infection. Children with symptoms of wheezing and/or eczema during first year of life were therefore excluded from the multivariate analyses (not in the paper). After this exclusion, the positive associations remained for doctor’s diagnosis of rhinoconjunctivitis (OR 1.78, 95% CI 1.09-2.91) and doctor’s diagnosis of asthma (OR 2.17, 95% CI 1.52-3.09), while all other associations between use of antibiotics and allergic disease remained or became non-significant.

We also excluded children with symptoms of wheezing and/or eczema during first year of life from the multivariate analyses on antipyretic use and allergic disease (not in the paper). After this exclusion the positive association between antipyretic use and current atopic eczema symptoms became non-significant (OR 1.42, 95% CI 0.97-2.07), while the positive association with doctor’s diagnosis of asthma became of borderline significance (OR 1.44, 95% CI 1.00-2.09).

We also conducted analyses where the health outcomes were combined with atopic sensitization to study IgE-mediated disease. These analyses were based on children who provided a blood sample and included about 30% of all children with question-
naire responses. In addition, the risk of any doctor’s diagnosis of allergy was studied in relation to the anthroposophic lifestyle factors. We found increased risks for antibiotic use (OR 1.94, 95% CI 1.58-2.38) and for antipyretic use (OR 1.23, 95% CI 1.01-1.51) during the first year of life, but no association for consumption of a biodynamic diet (OR 0.97, 95% CI 0.76-1.24).

4.1.3 Measles infection and measles vaccination

To be included in the analyses in paper II children must have completed the questionnaire, including the questions on measles vaccination and measles infection. A total of 2353 children were excluded due to incomplete answers (“do not know” or missing) to any of these questions. Thus, the analyses were based on 12540 children, including 3378 with blood samples.

The prevalence of measles vaccination and measles infection differed between the countries and different child groups in the PARSIFAL study. In total, 73% of the children were vaccinated against measles and 20% had had measles vaccination. Figure 8 shows the prevalence of measles vaccination and measles infection in the different countries.

Figure 7. Prevalence of measles vaccination and measles infection in all child groups in the PARSIFAL study (N=12540).
The Netherlands had the highest prevalence of measles vaccination (77%) regardless of group belonging, while in Austria and Switzerland it was lower. The lowest prevalence of measles infection was observed in Sweden (13%), whereas in Austria and Switzerland the prevalence was relatively high (23% and 26%, respectively). Measles vaccination was least common among the Steiner school children (26%), and there were no significant differences between the other groups. Steiner school children also reported the highest prevalence of measles infection (33%).

Measles vaccination is generally given in combination with mumps and rubella vaccines. In our data, 8206 (90%) of the children reporting measles vaccination also reported vaccination against mumps and rubella. In total, 972 children (8%) reported to have had both measles vaccination and measles infection.

To be vaccinated against measles, but not having had measles infection, was positively associated with rhinoconjunctivitis (current symptoms and doctor’s diagnosed), while being infected with measles, but not having received measles vaccination, was inversely associated with sensitization against food allergens (Fx5) compared to neither have been vaccinated nor infected with measles. In the subset of children with blood samples, we observed tendencies toward inverse associations between measles vaccination, infection, or both, and atopic sensitization (at allergen-specific IgE level of ≥0.35 and ≥3.5 kU/L). Similar results were observed when inhalant allergens (Phadiatop) and food allergens (Fx5) were analyzed separately. When the analysis was based on measles, mumps, and rubella (MMR) vaccination combined, the results remained the same.

It should be noted that a majority of unvaccinated children who never had measles infection (i.e., the reference category), as well as those with measles infection, were Steiner-school children (70% and 79%, respectively).

To reduce bias caused by disease-related modification of exposure (reverse causation), mainly regarding vaccination, we excluded children who reported symptoms of wheezing and/or eczema during the first year of life. The numbers in the symptom groups were small and, therefore, we performed analyses of measles vaccination and measles infection, respectively, combining all outcomes in the following two groups; any allergic symptom and any diagnose of allergy by a doctor (figure 8).

In analyses of all children, we observed inverse associations between measles vaccination and measles infection, respectively, and atopic sensitization, although statistically significant only for measles infection in relation to allergen-specific serum IgE level of ≥3.5 kU/L (figure 8). There were no significant associations between measles vaccination or infection and any allergic symptom or any diagnosis of allergy by a doctor. After excluding children with symptoms of wheezing and/or eczema during the first year of life, measles infection was inversely associated with any allergic symptom and any doctor’s diagnose of allergy, respectively, whereas the association with atopic sensitization was attenuated (figure 8). There was no clear heterogeneity in results when the four study groups were analyzed separately; however, these analyses had low statistical power (data not shown).
Figure 8. The associations between measles vaccination and measles infection and any allergic symptom/doctor’s diagnosis analyzed in a multivariate model, in all children (N=12540) as well as in children with blood samples (n=3378) and after excluding children with early symptoms of wheezing and/or eczema (N=11787, n=3167). The reference groups were children who were not vaccinated against measles, or who never had measles infection, respectively.

To increase the specificity of symptoms/doctor’s diagnosis in relation to allergic disease, analyses were performed combining these with atopic sensitization. Inverse associations were observed between measles vaccination and measles infection, respectively, and having any allergic symptom or any doctor’s diagnose of allergy in combination with atopic sensitization, compared with non-sensitized children free from symptoms/doctor’s diagnoses, although statistically significant only for measles infection. Measles vaccination was positively associated with having any allergic symptom or a doctor’s diagnose without being sensitized. After exclusion of children with symptoms of wheezing and/or eczema during the first year of life, no association remained statistically significant.

The association between MMR vaccination and measles infection, respectively, and allergic disease was investigated among Steiner school and Steiner reference children (paper I). MMR vaccination was positively associated with rhinoconjunctivitis (current symptoms and doctor’s diagnosis), while measles infection was inversely associated with IgE-mediated atopic eczema (current symptoms and doctor’s diagnosis). After excluding children with early symptoms of allergy, no associations were observed for MMR vaccination or measles infection and allergic disease.
4.2 THE BAMSE STUDY

4.2.1 Prevalence of allergic disease

At 8 years of age, the prevalence of asthma, allergic rhinitis, and eczema were 6.3%, 13.3%, and 16.3%, respectively. The prevalence among the children included in our analyses of fruit and vegetable consumption, as well as multivitamin supplementation, was somewhat higher (7.4%, 15.4% and 17.6%, respectively), but did not differ significantly from that of the cohort as a whole. Figure 9 shows the prevalence of rhinitis, asthma and eczema, also in combination with sensitization against any common inhalant or food allergens, or sensitization against birch pollen in particular. The prevalence of atopic sensitization against common food or inhalant allergens was 33%.

Figure 9. Prevalence of allergic disease among 8-year old children in the BAMSE cohort (N=2447), combined with sensitization against common inhalant and/or food allergens (Phadiatop and/or fx5, ImmunoCap system, Phadia AB, Uppsala, Sweden), and against birch pollen in particular.

4.2.2 Fruit and vegetable consumption

To be included in the analyses of paper III, answers on the questionnaires at baseline, and 8 years were required, together with a completed FFQ at 8 years of age (where the questions on fruit and vegetable consumption had to be completed) as well as information on atopic sensitization at 8 years of age. In total, 2447 children (51% boys and 49% girls) fulfilled these criteria.
The average consumption of fruit was 1.7 times per day (SD ±1.2), while vegetables were consumed on average 2.5 (SD ±1.5) times per day. Fruits consumed most frequently where apples/pears (25%), citrus fruits (9%), and bananas (8%), while cucumber (23%), carrots (15%), and tomatoes (13%) where most consumed among vegetables.

Total fruit and vegetable consumption was divided into quartiles. The distribution of potential confounding factors was evaluated and we observed statistically significant relations between sex, maternal age, socioeconomic status, maternal smoking during pregnancy and/or at baseline as well as iso-BMI and total fruit or vegetable intake. Similar results were observed when fruit and vegetable intake was investigated separately.

The associations between total fruit and vegetable consumption, respectively, and allergic disease were analyzed. Total fruit intake was inversely associated with allergic rhinitis (OR for highest vs. lowest quartile 0.56, 95% CI 0.41-0.78, \( P \) for trend <0.001), while no statistically significant association was apparent for total vegetable intake. Food related allergic symptoms for at least one of 6 pre-specified fruits or vegetables (apple, peach, kiwi, avocado, banana, fresh carrot, or “other”) was reported by 238 children (table 1). These children were excluded from the analyses since they might have changed their diet because of allergic symptoms. After exclusion of these children, the association between total fruit intake and allergic rhinitis became non-significant (OR 0.76, 95% CI 0.52-1.11, \( P \) for trend 0.216).

Of the 238 children who reported food related allergic symptoms, 55% were sensitized against birch pollen at 8 years of age. The prevalence of rhinitis (52%), asthma (22%), eczema (37%), and sensitization (66%) was considerably higher in this group than among the rest of the study population.

Table 1. Number of children who reported different food related allergic symptoms at 8 years of age (n=238). In this question, it was possible to tick more than one box.

<table>
<thead>
<tr>
<th>Itchy eyes and/or runny nose</th>
<th>OAS1</th>
<th>Wheezing</th>
<th>Diarrhea or vomiting</th>
<th>Eczema</th>
<th>Urticaria</th>
<th>Excluded due to symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>22</td>
<td>83</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Peach</td>
<td>4</td>
<td>38</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Kiwi</td>
<td>11</td>
<td>78</td>
<td>0</td>
<td>3</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Avocado</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Banana</td>
<td>0</td>
<td>15</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fresh carrot</td>
<td>10</td>
<td>51</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Other, what? (^2)</td>
<td>4</td>
<td>31</td>
<td>5</td>
<td>7</td>
<td>33</td>
<td>27</td>
</tr>
</tbody>
</table>

\(^1\) OAS=Oral Allergy Syndrome. This is a condition characterized by IgE-mediated immediate allergic symptoms restricted to the oral mucosa, which involves itching and vascular edema of the lips, tongue, palate and pharynx, with a sudden onset \(^5\).  
\(^2\) Citrus fruit, strawberry, and tomato were the most commonly listed foods.
When individual or groups of fruit or vegetables was analyzed the strongest and most consistent inverse associations were observed between consumption of apples/pears, as well as carrots, and allergic rhinitis, asthma, or atopic sensitization (figure 10). Fruit juice was not associated with any allergic disease (data not shown).

Figure 10. The associations between consumption of apples/pears, onions/leek, carrots, as well as legumes, and allergic disease and atopic sensitization in 8 year old children, in the BAMSE cohort. Results are presented for all children (N=2447), and when children with food related allergic symptoms are excluded (n=2209).
After excluding children with food related allergic symptoms, all associations became non significant, except the associations between onions/leeks and allergic rhinitis (OR for highest vs. lowest tertile 1.59, 95% CI 1.13-2.25, \( P \) for trend 0.010) and between legumes and asthma (OR for highest vs. lowest tertile 0.61, 95% CI 0.38-0.98, \( P \) for trend 0.035).

To distinguish between IgE-mediated and non-IgE-mediated disease, each outcome was combined with atopic sensitization. For intake of total fruit, apples/pears, and carrots, the inverse associations were restricted to IgE-mediated disease, while no statistically significant associations were observed for non-IgE-mediated rhinitis, asthma, or eczema. Total vegetable consumption was not associated with any IgE-mediated disease (data not shown). After exclusion of children who reported food related allergic symptoms all associations became non significant.

Children sensitized to birch pollen may develop hypersensitivity reactions to certain foods, for example apples or carrots, which may lead to avoidance of these food items. In our study, 16% of all children were sensitized to birch pollen at 8 years of age, corresponding to 46% of children with atopic sensitization, 66% with allergic rhinitis, 45% with asthma, and 28% with eczema. Total fruit consumption was inversely associated with sensitization to birch pollen (OR for the highest vs. lowest tertile 0.55, 95% CI 0.42-0.73). This inverse association persisted after exclusion of children who reported food related allergic symptoms (OR for the highest vs. lowest tertile 0.69, 95% CI 0.50-0.96). No association was found between total vegetable consumption and sensitization to birch pollen (OR for the highest vs. lowest tertile 0.89, 95% CI 0.64-1.24).

### 4.2.3 Vitamin supplementation

To be included in the analyzes of paper IV, answers on the questionnaires at baseline and the 8-year follow-up were required, together with information on vitamin supplement intake at 8 years of age (from the FFQ) and information on atopic sensitization at 8 years of age. A total of 2423 children fulfilled these criteria.

In total, 40.3% of the children reported to ever have used any type of vitamin supplement during the past 12 months. The most frequently used vitamin supplements are presented in figure 11. Multivitamins, with or without minerals, were the most consumed vitamin supplements (34.5%). On average, the children had started to use multivitamins when they were 5.7 years old.

The association between intake of any vitamin supplement or multivitamin and allergic disease and atopic sensitization was analyzed. Multivitamins with minerals and multivitamins without minerals yielded similar ORs and was therefore combined into one exposure, and called multivitamins.
Figur 11. Current intake of vitamin supplements during the past year in 8-year old children in the BAMSE cohort (N=2434 children).

There was no statistically significant association between current use of any vitamin supplement or multivitamins and asthma, allergic rhinitis, eczema, or atopic sensitization at age 8 y (figure 12). Further adjustment for early symptoms of wheeze or eczema had no major influence on the observed ORs.

Figure 12. The associations between current intake of any vitamin supplements or multivitamins and allergic disease and atopic sensitization at 8 years of age in the BAMSE cohort (N=2423).
In table 2, age at first use of multivitamins in relation to allergic disease and atopic sensitization was investigated among children who used multivitamins during the past 12 months. Multivitamin supplementation before or at 4 years of age had an inverse association with sensitization to food allergens (OR 0.61, 95% CI 0.39-0.97), as well as tendencies toward inverse associations with allergic rhinitis (OR 0.62, 95% CI 0.38-1.03). These results persisted when early symptoms of wheeze and eczema were added to the model (OR 0.60, 95% CI 0.37-0.96 for sensitization to food allergens; OR 0.59, 95% CI 0.34-1.00 for allergic rhinitis).

Table 2. The association between age at first use of multivitamins and allergic disease at 8 years of age among children in the BAMSE cohort (n=2423), calculated with a multivariate model.

<table>
<thead>
<tr>
<th></th>
<th>Age at first use of multivitamins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
</tr>
<tr>
<td>Asthma</td>
<td>1.0</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>1.0</td>
</tr>
<tr>
<td>Eczema</td>
<td>1.0</td>
</tr>
<tr>
<td>Sensitization</td>
<td>1.0</td>
</tr>
<tr>
<td>Inhalant allergens</td>
<td>1.0</td>
</tr>
<tr>
<td>Food allergens</td>
<td>1.0</td>
</tr>
</tbody>
</table>

In contrast, we observed non-significant associations with allergic rhinitis (OR 1.21, 95% CI 0.91-1.60) and eczema (OR 1.24, 95% CI 0.95-1.63) among children who started to consume multivitamins at or after 5 years of age. These association were somewhat attenuated after adjustment for early symptoms of wheeze and eczema (OR 1.08, 95% CI 0.81-1.45 for allergic rhinitis, OR 1.14, 95% CI 0.86-1.51 for eczema).

To distinguish between IgE-mediated and non-IgE-mediated disease, each outcome was also analyzed in combination with atopic sensitization to inhalant or food allergens. The results for asthma, allergic rhinitis, and eczema in combination with atopic sensitization resembled those described above.
5 DISCUSSION

5.1 MAIN FINDINGS

5.1.1 Diet

The dietary exposures that have been studied in relation to allergic disease in this thesis are consumption of a biodynamic diet (paper I), fruit and vegetable intake (paper III), and multivitamin supplementation (paper IV).

An association between fruit and vegetable intake, as well as multivitamin supplements, and allergic disease is biologically plausible because of the antioxidant and immunomodulatory properties of certain vitamins and minerals in foods. A postulated hypothesis is that antioxidants, because of their ability to quench free radicals, prevent chain reactions that could result in lipid per-oxidation and damage to cell membranes, which in turn could cause inflammation. Some studies show that biodynamic and organic foods contain more antioxidants, especially vitamin C, than conventional foods.

Biodynamic diet

Food and agricultural production certified as organic is a system that excludes the use of synthetic inputs, such as synthetic fertilizers, and pesticides, preventive veterinary drugs, genetically modified seeds and breeds, most preservatives, additives and irradiation. Biodynamic production is derived from the organic agriculture, and in addition it strives for a healthy eco-system within the farm.

We investigated the association between consumption of a biodynamic diet, compared to a conventional diet, and allergic disease in Steiner school and Steiner reference children, and did not observe any association. Different types of diets have been very sparsely investigated in relation to allergic disease, and there are very few studies that evaluated the effects of consumption of a diet based on biodynamically vs. conventionally grown foods in relation to allergic disease. In the study by Alm and colleagues the association between consumption of a diet mainly based on organically or biodynamically grown foods and allergic disease in children was investigated. They found that this kind of diet was associated with a lower prevalence of atopic sensitization, however, they could not separate this effect from other possible protective exposures due to the homogenous child group. Kummeling and colleagues studied the association between consumption of an organic diet and allergic disease in 2-year old Dutch children, and found that consumption of organic dairy products could have a protective effect on eczema, but the consumption of organically produced meat, fruit, vegetables, eggs or the proportion of organic food products within the total diet, were not associated with the development of wheeze, eczema or atopic sensitization.

Overall, the evidence does not seem sufficient to conclude that a biodynamic or organic diet is associated with a reduced risk of allergic disease in children.
An inverse association was observed between total fruit consumption and allergic rhinitis at 8 years of age. When individual food items were analyzed separately, the strongest and most consistent associations were observed for apples/pears and carrots, which were inversely associated with most of the studied outcomes. To evaluate potential disease-related modification of consumption, children who reported food related allergic symptoms were excluded from the analyses. After this exclusion, the majority of the observed inverse associations in all analyses became non-significant, suggesting that disease-related modification of consumption, that is, the child’s diet changed due to allergic disease/symptoms, influenced our results.

Most previous epidemiological studies of the association between fruit and/or vegetable intake and allergic disease in children have reported of a protective effect\textsuperscript{35, 63-69}. The associations between fruit intake and allergic rhinitis observed in our study before exclusion of children with food related allergic symptoms are in line with these results. Beside these inverse associations, also increased risks\textsuperscript{70, 71} or no associations\textsuperscript{72, 73} have been observed when fruit and/or vegetable intake have been studied in relation to allergic disease in children. A majority of the studies on fruit and/or vegetable intake in relation to allergic disease are cross-sectional\textsuperscript{35, 63-69, 71, 72}.

Only two studies have considered the potential problem with reverse causation. Kim and colleagues observed an increased risk of doctor’s diagnosed asthma in Swedish 5-14 year old children who consumed more fruits. When children who reported food allergy or intolerance was excluded from the analyses the association with asthma persisted\textsuperscript{71}. Wijga and colleagues excluded all children with cow’s milk allergy when studying food intake and development of allergy in 2-3 year old Dutch children, but no exclusion was made for children who eventually avoided fruit or vegetables. Despite this, they reported no association between fruit or vegetable intake and allergic disease\textsuperscript{73}.

We did not find any associations between total vegetable consumption and allergic disease, but single vegetables (onions/leeks and legumes) were significantly associated with allergic rhinitis and asthma, respectively. The worldwide ISAAC study investigated the consumption of vegetables, based on food balance data, in relation to allergic disease in children\textsuperscript{22}. They found that vegetable intake was inversely associated with current wheeze and atopic eczema. Since data on food consumption was based on food balance data that provide direct information about the availability of foods, but only indirect information about dietary intake, the result must be interpreted with caution.

Most studies on the relation between fruit or vegetable consumption and allergic disease report inverse associations, but a majority of these did not consider reverse causation. Our results were probably affected by disease-related modification of consumption, because of the high prevalence of birch pollen sensitization in the study population.
Multivitamin supplementation

Supplementation with multivitamins was investigated in relation to allergic disease in 8-year old children. Our results show no association between current use of multivitamins and risk of allergic disease, but suggest that supplementation with multivitamins during the first years of life may reduce the risk of allergic disease at school age. These results persisted after disease-related modification of exposure was taken into account.

There is very limited evidence on multivitamin use in relation to allergic disease in children. Milner and colleagues observed an increased risk of asthma among 3-year old black infants who consumed multivitamin supplements during the 6 first months of life, but they could not find any association between multivitamin use at 3 years of age and asthma at 3 years of age in either black or nonblack children. However, in a Norwegian study, intake of vitamin supplements during the first year of life was associated with a borderline significant increased risk of sensitization at school age\textsuperscript{35}. The reason for the apparently discrepant results might depend on differences among studies in composition, dose, and duration of the supplementation. Another potential important factor is the child’s nutritional status when taking the supplements.

The results from studies on vitamin supplementation among children and allergic disease appear inconsistent and too few studies have been performed on this topic to allow any conclusions on causal relationships.

5.1.2 Lifestyle

Several factors related to lifestyle have been studied in relation to allergic disease in this thesis. The anthroposophic lifestyle has mostly been in focus because of its many differences from conventional (i.e. Western) lifestyle. Certain features of the anthroposophic lifestyle of particular interest in relation to development of allergic disease include a low use of antibiotics and antipyretics, restrictive use of MMR vaccination and positive attitude to measles infection.

Use of antibiotics

The use of antibiotics in children has become more common during the past decades. This increase has coincided with the increased prevalence of allergic disease among children, which has lead to the hypothesis of a causal association\textsuperscript{74}. It is biologically possible that antibiotics could have an effect on the development of allergic disease, for example, because of an influence on the intestinal microflora. The intestinal microflora is a major factor driving the maturation of the immune system in newborns\textsuperscript{75}, and therefore it is plausible that use of antibiotics might affect this development negatively\textsuperscript{76}. Besides the respective interactions between antibiotics and bacteria and between the immune system and bacteria, both experimental and clinical research show that antibiotics also could interact directly with the immune system (for example on phagocytic function, chemotaxis and lymphocyte activities)\textsuperscript{76,77}.

Our analysis on Steiner school children and their referents (paper I) show positive associations between antibiotic use and all allergic diseases studied, except atopic sensitization, even after taking reverse causation into account. Another study on the
association between antibiotic use and allergic disease in Steiner school children in New Zealand concluded, in line with our results, that antibiotic use during first year of life may be associated with an increased risk of asthma, but they observed no associations between antibiotic use and the development of eczema 78.

Most previous studies investigating the association between use of antibiotics and allergic disease reported that antibiotic use, especially early in life, is associated with an increased risk of respiratory allergic disorders. Similar results were reported from a large meta-analysis by Marra and colleagues, where most studies on antibiotic use in relation to childhood allergic disease between 1966 and 2004 were included (four prospective and five retrospective studies) 74.

The observed relations between antibiotic use and respiratory allergic disease could represent a causal association; however reverse causation may also contribute, that is, if children with asthma symptoms received antibiotics on the presumption that they had a bacterial respiratory infection. Moreover, respiratory infections may act as confounders in the association between antibiotic use and allergic disease. In a Dutch birth cohort, Kummeling and colleagues accounted for reverse causation when they excluded children with wheezing during their first 6 months of life. After that they observed that exposure to antibiotics early in life increased the risk of recurrent wheeze in 2-year old children 79. Droste and colleagues excluded children with asthma or asthma-like symptoms during first year of life and concluded that children who received antibiotics early in life have an increased risk of developing asthma and allergic diseases, compared to children who did not receive antibiotics 80.

Other studies adjusted for LRIs (lower respiratory tract infections) or specific antibiotic treatment against LRIs. Illi and colleagues, did not observe any association between antibiotic use and wheezing in 7-year old children after having excluded antibiotic use for LRIs 81. In a study by Wickens and colleagues they concluded that the positive associations found between antibiotic use and allergic disease might be due to confounding by chest infections at an early age when asthma may be indistinguishable from infection 82. Celedon and colleagues adjusted for presence of lower respiratory tract infections (LRI) and the number of visits to the physician during first year of life, and concluded that antibiotic use is not associated with asthma development, but rather suggests that frequent antibiotic use in early life is more common among asthmatic children.83

There is today no clear evidence for the relation between antibiotic use and the development of allergic disease in children. It seems like most studies that considered confounding from LRIs did not observe any associations between antibiotic use and allergic disease. The fact that we found an increased risk of allergic disease after antibiotic use could be influenced by the fact that most children who did not use antibiotics (the reference category) were Steiner school children, who have a lower prevalence of allergic disease in general 43. In the PARSIFAL study we had no data on what kind of antibiotics that was used or the underlying infection it was prescribed for. Therefore there was no possibility to adjust for these potential confounding factors.

Use of antipyretics

The increased use of antipyretics over the past 50 years has coincided with the rise in prevalence of asthma worldwide 84. This has brought about investigations of all sorts of antipyretics in relation to the development of allergic disease in children. Possible
mechanisms explaining a role of antipyretics in asthma include oxidative stress and depletion of pulmonary glutathione. In addition, decreased glutathione may affect the development of asthma by altering antigen recognition towards favoring Th2 over Th1 cytokines.\textsuperscript{85, 86}

The association between antipyretic use and allergic disease is supported by a reported dose-response relation between paracetamol intake and asthma severity in young adults.\textsuperscript{87} Moreover, a strong correlation between paracetamol consumption and the incidence of atopic diseases in children has been reported.\textsuperscript{88} The findings by Kanabar and colleagues suggest that acetaminophen use in children is associated with an increased risk for wheezing.\textsuperscript{89} A systematic review and meta-analysis of studies of the relation between Acetaminophen use and the risk of asthma published between 1980 and 2008 showed that children exposed to Acetaminophen had an increased risk of asthma and wheezing.\textsuperscript{90} In the ISAAC study, data from 31 countries worldwide included the use of acetaminophen among infants and was associated with a 46% increased risk of current asthma symptoms at the age of 6 to 7 years. Increased risks were also observed for rhinoconjunctivitis and eczema, suggesting that the association extends to these related allergic conditions. It is not known to what extent reverse causation might have contributed to the association between acetaminophen and asthma.\textsuperscript{84}

The growing literature relating antipyretic use with respiratory conditions must be viewed with some caution. There is always the risk of confounding by the condition that leads to the use of the medication, but since antipyretics is used for many different conditions it is hard to adjust for the underlying condition. Further, the type of antipyretics used may vary a lot. We observed in paper I that the use of antipyretics during first year of life was positively associated with doctor’s diagnosis of rhinoconjunctivitis and current atopic eczema symptoms, but after excluding children with allergic symptoms during first year of life the positive associations became non-significant or of borderline significance. This could suggest that reverse causation affected our results.

**MMR vaccination and measles infection**

The occurrence of many types of childhood infections has decreased markedly during the past decades because of better hygiene and vaccinations, which has coincided with the increase of allergic disorders.\textsuperscript{51} This suggests that certain infections might have a role in the development of allergy. Infection with the measles virus may have an immune-suppressive effect and might affect the development of allergic disease. On the other hand, immunization against measles may also influence the development of allergic disease. One possible biological mechanism for this might be that the immune deviation is affected. Certain childhood infections and other environmental stimuli could switch the Th2 dominated cytokine profile, required at birth, to be Th1 dominated.\textsuperscript{3} It has been speculated that the absence of infections in childhood may cause the immune system to retain its Th2 cytokine profile, thus increasing the risk of allergy.\textsuperscript{3, 49}

We investigated the relation between measles vaccination and measles infection and allergic disease including all child groups in the PARSIFAL study. Overall, measles vaccination was associated with an increased risk of rhinoconjunctivitis (current symptoms and doctor’s diagnosis), while measles infection was inversely associated with sensitization to food allergens.
To minimize any effect from reverse causation children with early symptoms of wheezing and/or eczema were excluded from the same analyses. After this exclusion, measles vaccination was not associated with allergic disease, while an inverse association between measles infection and any allergic symptom or doctor’s diagnose was observed. The change in result after exclusion of children with early allergic symptoms may be a result of disease-related modification of exposure, that is, parents of children with early symptoms of allergy avoided or postponed measles vaccination, and perhaps also measles infection, which might be the case among certain anthroposophic parents.

However, previous studies on the impact of measles infection on allergic disease have shown conflicting results \(^{45-47, 50, 52, 54, 91-96}\). The timing of infection \(^{54}\), differences in outcome definitions, as well as methodological limitations might be of importance for the apparently discrepant findings \(^{97}\). Measles vaccination has also been associated with the development of allergic disease, but the evidence seems inconsistent \(^{46, 48-53, 92, 95, 98}\). We found six studies that assessed exposures before outcomes. One study observed an increased risk of asthma after MMR vaccination \(^{51}\), whereas another reported an increased risk of atopic dermatitis after either measles vaccination or infection \(^{46}\). Some studies did not observe any association between measles infection and allergic disease \(^{52, 54}\), or between measles vaccination and allergic disease \(^{48, 52, 53}\).

Overall, the results from studies on measles infection appear inconsistent. If there is a possible protective effect from measles infection on allergy in children it should be weighed against the fact that measles sometimes is a very serious disease with rare, but lethal complications, for example encephalitis \(^{99}\).

### 5.2 Methodological Aspects

#### 5.2.1 Study design

The PARSIFAL study is a cross-sectional multicentre study that focuses on two groups of children who have shown a lower prevalence of allergic disease and atopic sensitization. It is the largest study on Steiner school children ever made. Although the cross-sectional design is not optimal for elucidation of the temporal relation between exposure and outcome, since disease occurrence may have affected exposure or the reporting of exposure, this design is relatively common in studies on allergic disease in children. However, when data from cross-sectional studies are used to investigate associations between exposure and disease, the results must be interpreted with caution \(^{100}\).

The BAMSE study is a longitudinal birth cohort study. This study design is optimal when studying the temporal relation between exposure and disease because there is a possibility to collect information about exposures and then to follow the cohort and register the onset of symptoms/diseases when they appear. Even though the analyses in paper III and IV are cross-sectional, that is, information on exposure and symptoms/diseases came from the 8 year follow-up, we have used prospectively collected data for several exposures, such as heredity and socioeconomic status. The FFQ was included only at the 8 year follow-up and the dietary data from earlier questionnaires were sparse.
5.2.2 Random errors

In quantitative research, there is always the possibility for random error. Precision is defined as the absence of random error. The precision in a study mostly depends on the sample size, but also on the prevalence of exposure and disease, as well as on the quality of exposure and disease classification. In all papers included in this thesis we used 95% confidence intervals to provide information about the precision of the study. This means that if the data collection and analysis could be replicated many times, the confidence interval should include within it the correct value of the measure 95% of the time. A statistically significant result does not mean that chance can not have accounted for the findings, only that such explanation is unlikely. The large sample size of both the PARSIFAL and the BAMSE studies enhances the precision of the risk estimates (ORs). However, random error may be of more concern in subgroup analyses, such as in the analyses of onions/leek and legumes (paper III), use of multivitamins in early life (paper IV), as well as the analyses where the allergic disease was combined with atopic sensitization (paper I-IV). However, the consistency in the results for different health outcomes in our papers and the fact that our results mostly are comparable to other studies, indicate that chance alone is not a likely explanation for our findings.

5.2.3 Systematic errors

Systematic errors, also known as bias, is often classified into three categories: selection bias, information bias and confounding.

Selection bias

Selection bias is a systematic error in a study that stems from the procedures used to select subjects and from factors that influence study participation. It comes about when the association between exposure and disease differs for those who participate and those who do not participate in the study.

Selection bias is a potential problem in most epidemiologic studies. In the PARSIFAL study we had a relatively high response rate (69%), with an exception for the Netherlands where an outbreak of Foot- and Mouth Disease during the recruitment phase partly decreased the participation rate that was 50%. Moreover, similar proportions of the invited children from all child groups were included in all countries. We cannot exclude that non-response might affect the observed prevalence rates, but the prevalence of allergic symptoms among the reference children was comparable to a previous report covering the countries under study, speaking against any important selection bias.

Of greater concern is the selection of Steiner school children, because this process differed between the countries. In Sweden, the Netherlands and Austria all children who attended a Steiner school and completed the questionnaire were included as Steiner school children. In Germany and Switzerland, the Steiner school children were selected from their answers on specific questions about anthroposophic lifestyle in the questionnaire. This procedure would have made the German and Swiss Steiner school children “more” anthroposophic compared to the Steiner school children from the other countries. If this had any effect on our results, it would have resulted in an overesti-
mation of the associations between exposures and outcomes in these analyses. Moreover, after a first sampling seven Steiner schools and one reference school, all located in Germany, were excluded because of low participation rate, which appeared to be due to lack of interest on the part of the school rather than refusal by individuals. However, the consistency in results between the countries speaks against any major effect of such bias.

The “healthy farmer effect”, i.e. a selective avoidance of farming by atopic families may also be a problem. The reported prevalence of asthma and/or rhinoconjunctivitis was lower among parents in the farm groups than in the other studied child groups. This might in part be explained by families with atopic disease leaving their farms, although we do not have supporting evidence. Another possibility is that farming protects against atopy not only among children but also among adults, or that the childhood protection once similarly gained by the parent generation extends into their adulthood.

There might be a selection bias in results based on blood sample data in the PARSIFAL study, because the prevalence of allergic disease tended to be higher among reference children who provided a blood sample compared with children who did not. To minimize this problem, we adjusted for having any doctor’s diagnosis or symptom of allergic disease, which resulted in only a small change of the OR. Considering also that this represents an over-adjustment, it speaks against a major effect by selection bias.

About 59-60% of the children from the baseline cohort were included in paper III and IV based on the BAMSE study. These children were highly representative regarding distribution of exposure factors such as sex, parental allergic disease, maternal smoking and breastfeeding. The prevalence of allergic disease was somewhat higher, but did not differ from the cohort as a whole at 8 years of age.

**Information bias**

Systematic error in a study can arise when the information collected from study subjects is incorrect. This might lead to misclassification of study subjects with regard to exposure or disease. Misclassification of subjects for either exposure or disease can be differential or non-differential. For exposure misclassification, the misclassification is non-differential if it is unrelated to the presence of disease. If the misclassification of exposure is different for those with and without disease, it is differential. Similarly, misclassification of disease is non-differential if it is unrelated to exposure, otherwise, it is differential.

There is always reason to believe that some misclassification of exposure can be present. The information of measles vaccination used in paper II was based on parental recall. Such information has been associated with both underestimation and overestimation in validation studies. The typical symptoms of measles infection, high fever and characteristic skin rash, are often distinct and appear in epidemics, which helps to make parental reports of measles reliable. However, in the Parsifal study 8% of the parents reported that their child had both been vaccinated against measles and had a measles infection. This may be explained by differences in vaccination coverage and year of introduction of the vaccine and recurrent measles epidemics. Some children
in or study presumably received only one dose of measles vaccine which makes it easier for vaccinated children to get infected \textsuperscript{99}. However, we can not exclude that a parental report of both measles vaccination and infection in the same child, at least partly, is explained by misclassification of measles vaccination or infection. It is unlikely that this misclassification is differential in relation to allergic disease. Therefore, this would not change the direction of the observed associations.

Dietary information collected in an epidemiologic study is often affected by measurement error \textsuperscript{104}. A common reason for this is memory failure. Despite this, the precision of the FFQ to classify frequency of food consumption is usually good \textsuperscript{105}. Misclassification of exposure might have affected the results on fruit and vegetable consumption and vitamin supplementation (paper III and IV), since parents of children might have found it difficult to remember the frequency of fruit and vegetable consumption or multivitamin supplementation during the past year. The parents were also asked when their child started to take the vitamin supplements. The diet of allergic children might actually have changed due to the allergic disease, why parents of allergic children may report dietary intake or multivitamin supplementation differently from parents of non-allergic children. This would lead to differential misclassification of exposure, which was partly taken into account when children who reported food related allergic symptoms were excluded. The association between multivitamin supplementation in early life and allergic disease did not change after adjusting for early symptoms of allergic disease, suggesting that this was not a major problem in these analyses.

In both the PARSIFAL study and the BAMSE study parental interpretation of the child’s symptoms might lead to misclassification of disease, but several health outcomes included a doctor’s diagnosis and/or serological analyses, which would decrease misclassification.

Confounding

Confounding is a central issue for epidemiologic study design. Confounding can be explained as a mixing of the effects, i.e. the effect of the exposure is mixed with the effect of another variable (confounder). For a variable to be a confounder it must be a risk factor for the studied disease and related to the exposure under study \textsuperscript{101}. In the analyses of paper I-IV confounders were either selected through a process where they changed the risk estimate by 10\% or more when included in the crude model, or they were generally recognized risk factors for allergic disease in children. Most variables fulfilled both criteria. Despite this systematic selection of confounders, there might still be an effect from residual confounding, that is, from other factors in the child’s diet, lifestyle, or environment that was not adjusted for in the analyses.

When we studied antibiotic use and allergic disease (paper I) we had no information on the type of antibiotics used or for what condition they were prescribed, which might have confounded these results. In similar studies that could adjust for these possible confounding factors, the associations between antibiotic use and allergic disease became non-significant \textsuperscript{81-83}.
Disease-related modification of exposure

When there is reason to suspect that the outcome precedes the exposure, disease-related modification of exposure (reverse causation) should be taken into account. This problem mostly occurs in cross-sectional studies, but even in prospective studies there may be a risk that certain characteristics related to the outcome, e.g. allergy among family members, may confound the association between exposure and disease. Disease-related modification of exposure has been discussed in several articles investigating behavioral factors, such as breastfeeding 106, pet ownership 107 and consumption of specific food items 108 in relation to allergic diseases. In these studies disease-related modification of exposure had an important contribution to the results.

There are different ways to deal with this dilemma, and it can be done at different stages of the study, for example, to collect data on exposures prospectively (design stage), or to group subjects according to age of exposure/outcome if that information is available (analysis stage), or to exclude subjects for whom the information on timing of the exposure/outcome is incomplete (analysis stage). In all papers in this thesis, we excluded or adjusted for children with early symptoms of allergic disease to try to reduce reverse causation. This exclusion often resulted in changes of the results. Disease-related modification of exposure seems to have influenced our results in paper II and III, where a majority of the associations disappeared after excluding a group of children who either had early allergic symptoms or reported food related allergic symptoms. In paper I the increased risks related to MMR vaccination and antipyretic use, as well as the inverse association related to measles infection, became non-significant, while in paper IV it had no major influence on the results.

When studying allergic diseases it is difficult to estimate the incidence rate. Because of the natural history of allergic disease, it is not known when the child became allergic. The allergic march often starts with eczema early in life, later on asthma and then rhinitis and these conditions are often accompanied by atopic sensitization. Atopic sensitization is measured with one blood sample or skin prick test, and therefore, it is almost impossible to determine exactly when the child became sensitized.
6 CONCLUSIONS

Based on the papers included in this thesis, in combination with the available scientific literature in the areas covered by these papers, the following conclusions can be drawn:

- The lower prevalence of allergic disease among Swedish Steiner school children could be confirmed in several European countries, and was related to a restrictive use of antibiotics and antipyretics. However, we can not exclude that there are other factors in the anthroposophic lifestyle that contributed to this lower risk.

- Measles infection is frequently found among Steiner school children and may have a protective effect on allergic disease in children. The scientific evidence on measles infection and allergy appears inconsistent, and any possible protective effects from measles infection must be weighed against the fact that measles infection is sometimes a very serious disease. Moreover, no associations were found between measles vaccination and allergic disease.

- We could confirm the inverse association between fruit consumption and allergic disease in children. However, our data also indicate that disease-related modification of consumption contributed to this association.

- Current multivitamin supplementation was not associated with allergic disease among school children. However, supplementation during the first years of life was inversely associated with sensitization to food allergens in our study. This result persisted also after considering disease-related modification of exposure. The cross-sectional nature of our data implies that the findings should be interpreted with caution.

- These studies show that disease-related modification of exposure (reverse causation) is an important factor to consider when studying allergic disease. This is especially important in cross-sectional studies, but the fact that the natural history of allergic disease could cause the same child to experience different allergic symptoms at different ages, suggests that disease-related modification of exposure may be a possible confounding factor also in prospective studies.
7 SVENSK SAMMANFATTNING

Förekomsten av allergi hos barn har ökat dramatiskt sedan 1900-talets andra hälft. Orsakerna till ökningen är oklara, men miljö- och livsstilsfaktorer relaterade till en västerländsk livsstil verkar vara av betydelse. Denna livsstil innebär en förändrad kost och färre infektioner i barndomen, samt en ökad användning av antibiotika, antipyretika och vaccinationer. Alla dessa faktorer har studerats i relation till allergi, men ingen av dem kan enskilt förklara den globala ökningen av allergisjukdom.


I analyser av de 4600 barn som går i Waldorfskola och 2000 kontrollbarn från PARSIFAL, kunde vi bekräfta den lägre förekomst av allergisjukdom som tidigare rapporterats hos svenska barn med antroposofisk livsstil. Användning av antibiotika och antipyretika var relaterat till en ökad risk för astma och eksem, medan enbart antibiotikaanvändning var kopplat till en ökad risk för hösnuva. Vi kan dock inte utelösa att andra faktorer i den antroposofiska livsstilen kan ha bidragit till resultaten.

Vi studerade också sambandet mellan mässlingsvaccination samt mässlingsinfektion och allergisjukdom bland alla barn i PARSIFAL-studien. För att undvika att allergisjukdomen förändrat exponeringen för mässlingsvaccination- eller infektion, uteslöts barn med tidiga symtom av allergi från analyserna. Därefter förelåg inga samband mellan mässlingsvaccination och allergisjukdom, medan barn som haft mässlingsinfektion hade en lägre risk för allergi.

I BAMSE-kohorten hade vi information om kostintag och allergisjukdom vid 8 års ålder för 2400 barn. Bland dessa barn var ett högt intag av frukt kopplat till en lägre risk för hösnuva. Sedan barn som var allergiska mot frukter eller grönsaker (till exempel äpplen eller råa morötter) uteslutits från analyserna, eftersom de allergiska symtomen kan ha påverkat deras kost, försvann de flesta samband. Intag av multivitaminer det senaste året påverkade inte risken för allergisjukdom, medan barn som började äta multivitaminer tidigt i livet hade en lägre risk för födoämnessensibilisering.

Sammanfattningvis visar våra studier att sjukdomsrelaterad förändring av exponeringen är en viktig faktor att ta med i analyserna då beteenden studeras i relation till allergisjukdom. Detta är framför allt viktigt i tvärsnittsstudier, men på grund av att allergisjukdomars naturliga utveckling kan innebära att samma person har olika allergiska symtom vid olika tillfällen i livet (till exempel eksem under spådbarnsåren, följt av astma och/eller hösnuva senare i barndomen), så kan sjukdomsrelaterad förändring av exponeringen även vara ett problem i prospektiva studier.
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