Prenatal and childhood nutrition and the development of asthma and allergy in children

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Prenatal and childhood nutrition and the development of asthma and allergy in children

Prenatale voeding en voeding van het kind en de ontwikkeling van astma en allergie bij kinderen

(met een samenvatting in het Nederlands)

Proefschrift

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Bon Accord

Contents

Chapter 1

General introduction

The main objectives of this thesis were to investigate associations between maternal diet during pregnancy, the child's diet and symptoms of asthma and allergy in children from two prospective birth cohorts in the Netherlands (PIAMA) and Aberdeen, UK (SEATON).

1.1 Background of asthma and allergic disease

Asthma is a chronic disorder that is characterised by recurrent symptoms of wheeze, breathlessness, chest tightness and cough, often associated with airway hyperresponsiveness, variable airflow limitation and chronic inflammation of the airways.[1] Asthma, together with allergic rhinitis, atopic eczema and food allergy are allergic diseases which have in common that they often involve type-I hypersensitivity to certain allergens: atopy. Atopy is a personal or familial tendency to become sensitized and produce IgE antibodies in response to ordinary allergen exposures such as house dust mite, grass and tree pollen, fungi, or food proteins.[1] Upon allergen exposure, antigen presenting cells interact with T-helper cells which differentiate into either T-helper 1 (Th1) or T-helper 2 (Th2) cells. Th1 cells produce cytokines which play an important role in host defence against pathogens, whereas Th2 cells produce cytokines which stimulate allergic inflammation by activation of eosinophils and the production of IgE. Cytokines produced by Th1 cells inhibit the growth and function of Th2 cells and vice versa. Once there are allergen-specific IgE antibodies adhered to mast cells and basophils, exposure to allergens can trigger the release of cytokines and mediators such as histamine, TNF-alfa, leukotrienes and chemokines which are responsible for the acute allergic symptoms (e.g. bronchoconstriction). The influx of inflammatory cells in the later phase of an allergic reaction causes inflammation of the airways, skin or nasal mucosa.[2,3] Diagnosis of asthma in small children is difficult, because they are not yet able to perform reliable lung function and bronchial hyperreactivity tests. Therefore, diagnosis of asthma is often based on the presence of (a combination of) respiratory symptoms like wheeze, dyspnea, chest tightness, cough and atopic sensitization.

1.2 Prevalence of asthma and allergy in children in the Netherlands and Aberdeen, UK

Since about the 1960's the prevalence of asthma and allergy has increased considerably.[4] Data from the International Study of Asthma and Allergies in Childhood (ISAAC) have shown that asthma is more prevalent in Westernized societies than in rural or developing regions. Highest prevalences of asthma symptoms were found in the UK, Australia, New Zealand and Ireland, whereas the lowest prevalences were found in Eastern European countries, Indonesia, Greece, China, Taiwan, Uzbekistan, India and Ethiopia.[5]

Internationally, the Netherlands is approximately in the middle of the list ranking the prevalence of these disorders.[6] Figure 1.1 shows the prevalence of doctor-

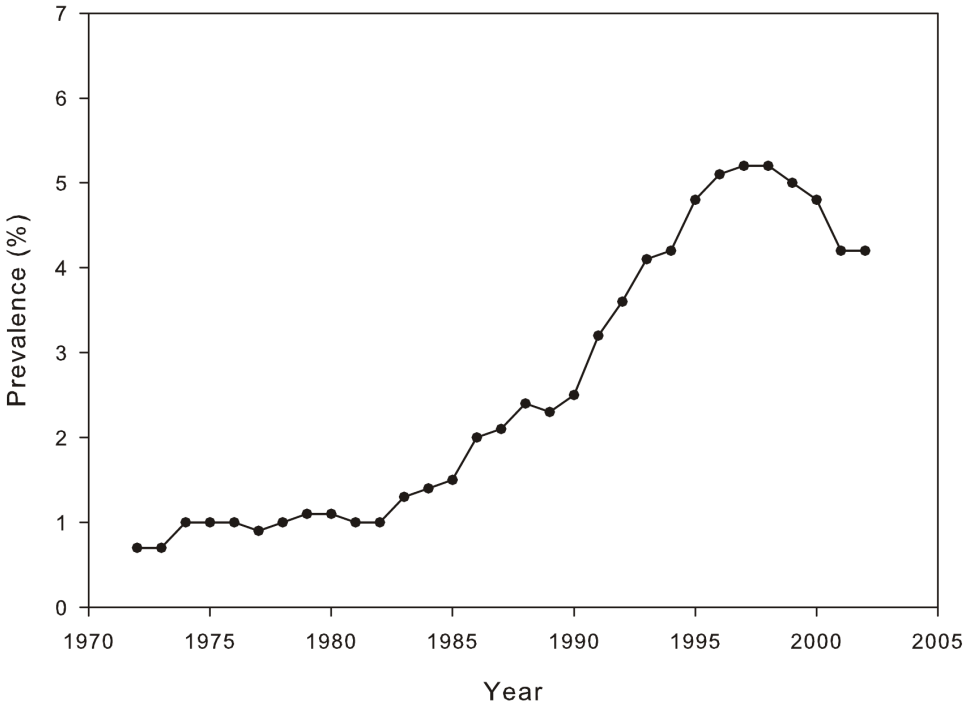

Figure 1.1: *Prevalence of doctor-diagnosed asthma in 0 to 14-year-old children in the Netherlands from 1972 to 2002. Figure is reproduced from data from general practice registrations of the Dutch Continuous Morbidity Registry Nijmegen (www.nationaalkompas.nl)* [7]

diagnosed asthma in 0 to 14-year-old children in the Netherlands from 1972 to 2002,[7] whereas figure 1.2 shows the results from four cross-sectional surveys of 9 to 12-year-old schoolchildren between 1964 and 2002 in Aberdeen, UK.[8] Prevalence of asthma in the Netherlands presented in figure 1.1 is considerably lower than the prevalence of asthma in Aberdeen, UK. Although the prevalence of asthma in the UK is higher than in the Netherlands, this difference is also caused by the fact that the data from the Netherlands has been retrieved from general practice registrations and not from a survey in the open population. The general practice registrations provide a fair reflection of the morbidity presented to a general practitioner, but not necessarily of the total morbidity in the open population because diseases are often underreported. Not everyone who has asthma symptoms consults his or her general practitioner.[9] Epidemiological population studies often use standardised questionnaires to measure aspects of asthma morbidity. However, a disadvantage is that the symptoms are not clinically interpreted by a doctor. Both sources are important to get a good impression of the actual prevalence data.[10]

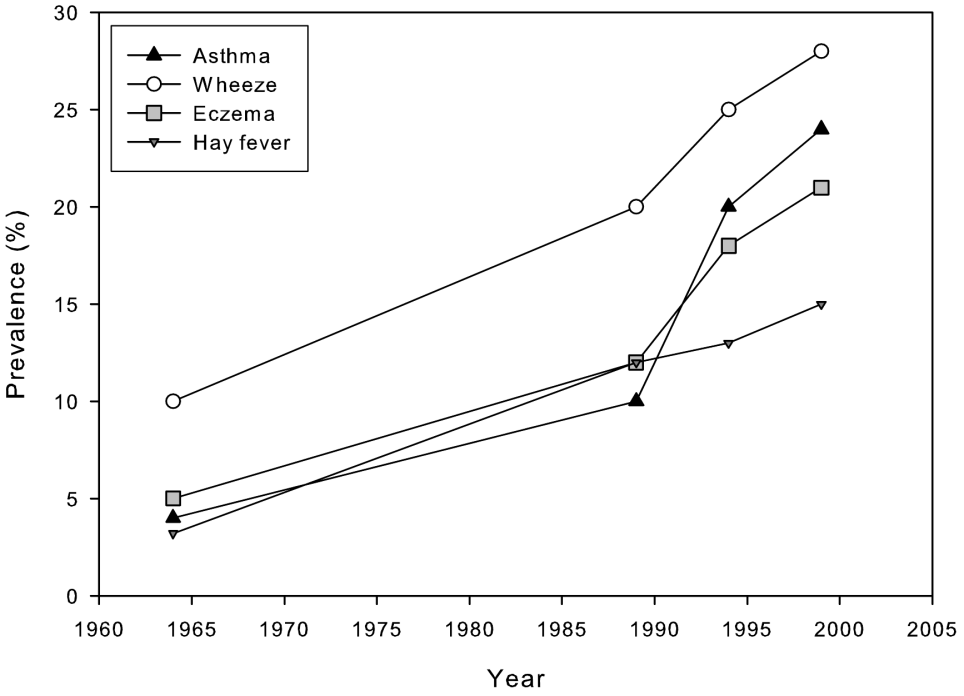

Figure 1.2: *Prevalence of asthma, wheeze, eczema and hay fever from 1964 to 1999 in Aberdeen, UK. Figure is reproduced from data reported in a paper of Devenny and colleagues* [8]

1.3 Risk factors for the increase in allergic disease

Although asthma and allergy have genetic determinants, the observed increase in asthma and allergy has occurred too rapidly for genetic changes to explain the increase. This, and the fact that the prevalence of asthma and allergy is much higher in the more affluent Westernized societies than in rural or developing regions, has led to consideration of several environmental and lifestyle determinants to explain the increase. First, it was thought that the major cause for the increase in asthma and allergies was the increased exposure to allergens because of carpet use, warmer houses and more time spent indoors. Exposure to allergens, especially in early life, produces atopic sensitisation leading to asthma and other allergic diseases.[11,12] Yet, the evidence that allergen exposure is the primary cause of childhood asthma is weak, and there is relatively little evidence that allergen exposure levels have changed over time.[13–15] A second well-known hypothesis to explain the increase in asthma and allergic disease is the so-called 'hygiene hypothesis'. This hypothesis, introduced by Strachan who found that the risk to develop hay fever was smaller with presence of older siblings,[16] proposed that the increase in allergic diseases is caused by a disrupted

balance in Th1/Th2 cell responses resulting from decreased microbial exposure due to immunisation, antibiotic use, water purification, reduced family size and improved personal and environmental hygiene.[17] Thirdly, the increase in asthma and allergy could be explained by a change in dietary habits. This hypothesis will be further discussed below.

1.4 The influence of diet on asthma and allergic disease

Increasing prosperity in the Western world after the Second World War, has led to large changes in diet. Since the 1960s of the last century our dietary habits have evolved from primarily consumption of locally produced and marketed fruit, vegetables, fish and meat to increased consumption of foods that are processed, modified, stored and transported over large distances. Furthermore, it has been suggested that the mineral content of fruit and vegetables has declined due to changes in cultivation, the use of fertilisers and the choice of species that can be more easily harvested or stored.[18]

Since the mid-eighties of the last century this prosperity associated change in diet has been linked to the increase in asthma and allergy, starting with an observation of Burney that regional table salt use was associated with asthma mortality.[19] Increased sodium intake could cause increased airway reactivity through potentiation of the electrogenic sodium pump in membranes of smooth muscle cells in the airways.[20] Two other important hypotheses relating the change in diet to the increase in asthma and allergic disease are the antioxidant and the lipid hypotheses described below.

1.4.1 The antioxidant hypothesis

In 1994, Seaton and colleagues[21] proposed that dietary changes leading to reduced intake of foods rich in antioxidants like vitamin C, E and β-carotene have led to a reduction in antioxidant defences, resulting in a shift of the antioxidant status of the whole population. A plausible mechanism could be that a reduced antioxidant defence mechanism in the airways leads to increased susceptibility to oxidant attack and airway inflammation, and thus an increase in prevalence of asthma.[21]

Nutrients such as vitamin E, selenium and flavonoids also have nonantioxidant properties related to asthma and atopic disease. Research has shown that these antioxidants could exert an effect on Th cells, that is, promoting Th1 cell differentiation by increasing Th1 cytokine production and/or inhibiting Th2 cell cytokine production.[22]

1.4.2 The lipid hypothesis

Interest in the influence of dietary fat intake on allergic disease started with an observation of a very low prevalence of asthma in Inuit populations, who have a high fish intake.[23] In 1997, Black and Sharpe[24] hypothesized that in industrialised countries

the increase in the prevalence of atopic diseases has been preceded and then paralleled by a fall in the consumption of saturated fat (animal fat) and oily fish (containing n-3 PUFAs) and an increase in the intake of polyunsaturated fat (margarine and vegetable oils containing n-6 PUFAs), resulting in a shift in the n-6/n-3 ratio of fatty acid intake.

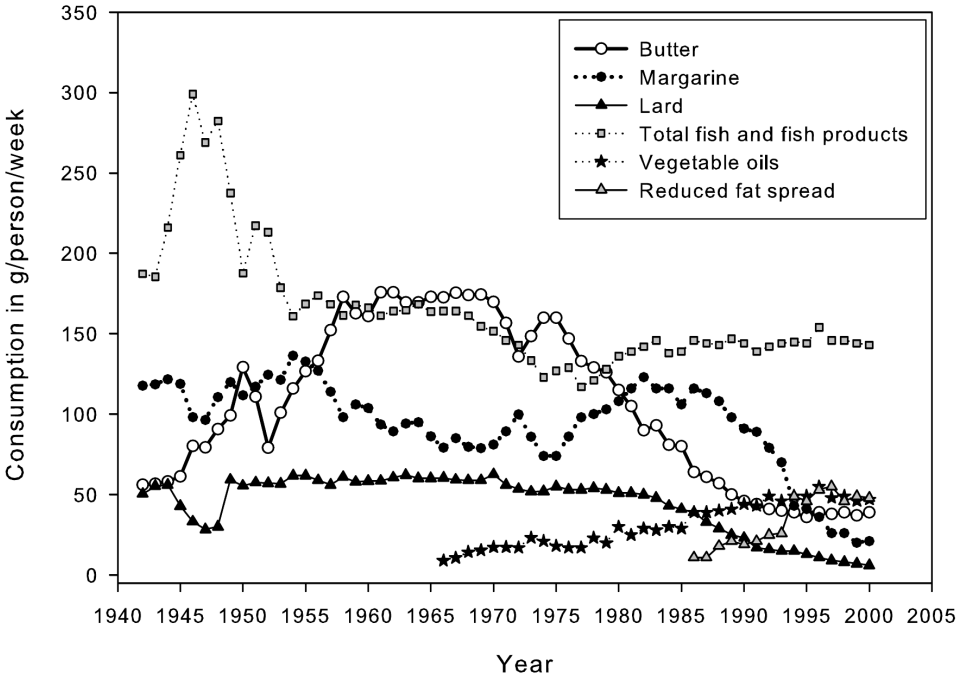

Figure 1.3: *UK consumption of foods rich in n-3 and n-6 PUFAs and saturated fat from 1942 to 2000* [25]

Figure 1.3 shows the consumption of foods rich in n-3 and n-6 PUFAs and saturated fat in the UK from 1942 to 2000. From this figure one can see that the picture that Black and Sharpe sketch is more or less true. Consumption of total fish and fish products has decreased in the sixties and seventies of the last century (due to decreased consumption of local fresh fish), but then slightly increased and stabilised (due to increased consumption of tuna, imported fish and shellfish). Furthermore, the intake of saturated fat from butter and lard decreased from the mid-seventies onwards, whereas the consumption of margarine and vegetable oils starts to increase at that time. From the end of the eighties onwards margarine has been replaced by reduced/low fat spread use.[25]

One of the proposed mechanism by which increasing dietary intakes of n-6 PUFAs from margarine and vegetable oils and decreasing intakes of n-3 PUFAs from oily fish

could promote asthma incidence is considering the critical role of PUFA as precursors of the eicosanoids.[24] Eicosanoids are a complex group of highly biologically active compounds with 20 carbon atoms produced by cells from released membrane PUFAs (in particular arachidonic acid, dihomo-γ-linolenic acid and eicosapentaenoic acid), and used as chemical messengers within the immune system (reviewed by Stulnig[26]). The most common dietary PUFAs are linoleic acid and α-linolenic acid, which are the parent fatty acids for the n-6 and n-3 PUFA families, respectively. They can be further metabolised in the body, but they share the same pathway, competing for the same set of elongases and desaturases. Linoleic acid is converted into arachidonic acid that can then be metabolised through cyclooxygenase and lipoxygenase enzymes to produce eicosanoids from the 2-series prostaglandins and thromboxanes and the 4-series leukotrienes, which are believed to be major mediators of asthmatic bronchostriction. 4-series leukotrienes have proinflammatory activity[27] and have been detected in the blood, bronchoalveolar lavage fluid and urine of asthmatics,[28] while the 2-series prostaglandin PGE2 is known to have immunomodulatory properties, promoting the Th2 phenotype associated with asthma and atopic disease. Black and Sharpe argued that increasing dietary intake of n-6 linoleic acid has resulted in increased arachidonic acid-derived eicosanoids (e.g. PGE2) production with a consequent increase in the likelihood of atopic Th2 sensitisation, asthma and atopic disease.[24] Conversely, alterations in arachidonic acid-derived eicosanoids have long been regarded as a primary mechanism to elucidate the immunomodulatory action of C20 PUFAs, particularly those of the n-3 series. Indeed, if eicosapentaenoic acid (EPA) replaces arachidonic acid in immune cell membrane phospholipids, it can inhibit hydrolysis of arachidonic acid from membrane phospholipids, and compete with arachidonic acid for cyclooxgenase and lipoxygenase enzymes. EPA-derived eicosanoids can oppose the effects of those derived from arachidonic acid by competing for receptor binding. Finally, EPA-derived eicosanoids have differing effects from those derived from arachidonic acid and are less effective.[29] However, the consequences of a shift in the n-6/n-3 ratio of fatty acid intake are undoubtedly more complex than outlined by Black and Sharpe. Although, eicosanoids were first regarded as a key link between fatty acids (particularly n-3 PUFAs) and immune function, the question as to whether and to what extent PUFAs alter the immune response via interference with eicosanoid production remains still unsolved. Indeed, both n-3 and n-6 PUFA can also modulate T-cell function directly through effects on cell membrane fluidity, in particular lipid rafts, cell signalling and gene expression.[26]

1.4.3 Maternal diet during pregnancy

According to the fetal programming theory or Barker hypothesis, adverse events during pregnancy like smoking, undernutrition, and infections can have a major impact on fetal development, which could result in long-term physiological and metabolic changes.[30,31] This concept has been proposed to play a role in the development of asthma and allergy as well. Development of airways and immune system mainly occurs during fetal life, several studies have found associations between indicators of impaired fetal growth such as low birth weight, and head circumference and asthma

and allergy in childhood.[32–34] Dietary factors that may influence the susceptibility to develop asthma and allergy have therefore been thought to exert their main influence through effects on airway and immune system development during fetal life. Studies have shown that maternal diet during pregnancy has the potential to modulate the risk of childhood asthma and allergy by affecting airway development and promoting Th-cell differentiation towards the Th2 cell type.[35]

1.5 Design of the PIAMA and SEATON birth cohort studies

1.5.1 The PIAMA birth cohort study

The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study, initiated in 1996, consisted of a natural history part to study the role of environmental and dietary risk factors for the development of asthma and allergy in childhood, and of an intervention part to evaluate the use of mite-impermeable mattress and pillow covers. Recruitment took place in 1996–1997. Before enrollment in the cohort, 10,232 pregnant women completed a validated screening questionnaire when visiting one of 52 prenatal healthcare clinics. Resulting from this screening, 2949 women were defined as atopic, while 7283 were defined as non-atopic. 2779 atopic women and 5083 non-atopic women were invited to participate in the study, of whom 1327 atopic women and 2819 non-atopic women agreed to participate and gave written informed consent. In the intervention part of the PIAMA study only children born to atopic mothers ('high-risk' children) were enrolled, whereas in the natural history part children of atopic as well as children of non-atopic mothers ('low-risk' children) were enrolled. The oversampling of non-atopic mothers in the natural history part of the study provides that the proportion of atopic and non-atopic mothers enrolled in the total study is the same as in the screened population (∼30%). Study children were born between summer 1996 and late fall 1997, the study started with 3963 newborn children because 183 participants were lost to follow-up before any data on the child had been obtained. Questionnaires were administered when the children were 3 months of age, and annually from 1 to 8 years of age. At 8 years of age, the children who were still participating in the study were invited for a medical examination including an interview, anthropometry, collection of blood samples to determine IgE antibodies, eosinophils, blood sugar and DNA, the more extensive examination additionally included measurement of NO in nasal cavities and exhaled breath (FeNO), measurement of lung function (FVC and FEV_1), MicroRint as indicator for airway resistance, a metacholine provocation test as indicator for bronchial hyperresponsiveness (BHR) and a skin prick test for allergy. Children from the intervention part, the 'high-risk' natural history part, and a random sample of the 'low-risk' natural history part, drawn at the beginning of the PIAMA study, were selected for more extensive follow-up including home visits, medical examination at 4 years of age, and the more extensive medical examination at 8 years of age. Further details on the study design have been published previously.[36]

4,112 of the 4,146 pregnant women completed the pregnancy questionnaire. Questionnaire data from age 1 to 8 years of age were obtained for 3817, 3740, 3694, 3563, 3518, 3473, 3373, and 3320 children respectively. In total, 2214 children have data on (parts of) the 8-year medical examination.

1.5.2 The SEATON birth cohort study

The Study of Eczema and Asthma To Observe the influence of Nutrition (SEATON) was set up to investigate associations between maternal diet during pregnancy and childhood asthma and allergic disease. Two thousand healthy pregnant women were recruited at a median gestational age of 12 (IQR 11–13) weeks, during their first visit of Aberdeen Maternity Hospital's antenatal clinic between October 1997 and April 1999. Pregnant women were recruited irrespective of asthma or atopic status, and an examination of a clinical database of all pregnant women attending the Maternity Hospital at the time demonstrated that the recruited women were representative of the local obstetric population apart from expected slight biases (socio-economic status, age, smoking history). At enrolment, a research nurse conducted a short interviewer-administered questionnaire which contained questions on demographic details, parity, expected date of delivery, height, weight, social class, smoking habit, and respiratory and allergic symptoms. A non-fasting venous blood sample was obtained and atopic status was assessed by skin prick tests for common allergens. At 32 weeks of gestation a semi-quantitative food frequency questionnaire (FFQ) (version 5.4 of the Scottish Collaborative Group FFQ) was mailed to the participating pregnant women. This questionnaire enquired about the consumption of 145 foods, and the use of vitamin and mineral supplements during the previous three months. Between April 1998 and December 1999, 1,924 singleton children were born to the 2,000 women. Thirty-four women gave birth to twins and were excluded from the study, and 42 women lost their babies through miscarriage, intrauterine death, stillbirth, or neonatal death. Follow-up of the original cohort was limited to these 1,924 singleton births. At delivery, maternal and cord blood samples were obtained for the measurement of antioxidant and lipid status, in a subsample of 224 neonates cord blood immunological studies were performed. Children were followed up by postal questionnaire at 6 months, 12 months, 2 years and 5 years of age. These questionnaires contained core ISAAC questions and enquired about symptoms of wheeze (in the presence or absence of a 'cold'), breathlessness, rhinitis, and doctor-diagnosed asthma, eczema, or hay fever, and possible determinants of asthma and allergy such as number of other children in the house, pets, and antibiotic use. Parents responding to the health questionnaire at 5 years were invited to complete an FFQ (version C1) to assess the dietary intake of the study child over the previous 3 months. This 121–item semiquantitative FFQ was based on the questionnaire used for the mothers in pregnancy but modified for preschool children by simplifying the response choices and changing the food list and portion sizes. Parents were also invited to bring the study child to the hospital for an assessment that included spirometry, skin-prick testing, measurement of bronchodilator response and measurement of exhaled nitric oxide (FeNO). Bronchodilator response and FeNO measurements were not included in the original study protocol

but were introduced for the last 510 and 262 children respectively.

1751 (91%) of the mothers satisfactorily completed the pregnancy FFQ. The response rates to the health questionnaire at 6, 12, 24 months and 5 years were 1,637 (85.1%), 1,512 (78.6%), 1,374 (71.4%), and 1,253 (62.7%) respectively. Dietary data at 5 year follow-up were obtained for 1,120 (89%) of the 1253 children who also had information on the health questionnaire. 797 (64%) children attended for hospital assessment. All of the 797 children attempted to perform spirometry; 639 (80%) were successful and 478 children were able to provide a prebronchodilator FEV_1 measurement. Of the 502 children who attempted post-bronchodilator spirometry, 383 (76%) were successful and 269 children were able to provide a post-bronchodilator measurement. Skin-prick reactivity and FeNO were determined in 700 and 167 children, respectively. The Grampian Research Ethics Committee approved the study and written parental consent was obtained for all parts of the study. Further details on the SEATON study have been published previously.[37,38]

1.6 Outline of the thesis

The number of epidemiological studies regarding nutrition and allergic disease has grown considerably over the last decades. Yet, the evidence is still conflicting. **Chapter 2** of this thesis contains a review of the epidemiological evidence regarding the relation between nutrition and allergic disease in children from the mid-eighties up until now.

Previous results from the SEATON birth cohort have shown associations between maternal nutrient intake during pregnancy (e.g. vitamin E, vitamin D and zinc) and cord blood mononuclear cell responses at birth, wheezing in the second year of life and wheeze, asthma, eczema, lung function and exhaled nitric oxide at 5 years of age.[37–40] To date, there are few reports relating maternal intake of specific foods during pregnancy and the subsequent development of childhood asthma and atopic disease. If associations with foods or food groups are identified, possible dietary recommendations to prevent childhood asthma or allergy are more ethically acceptable than supplementation with nutrients. In **chapter 3**, we investigate associations between maternal intake of individual foods and food groups (total fruit, citrus/kiwi fruit, apples, total vegetables, green leafy vegetables, pure fruit juice, whole grain products, total fish, total oily fish, total fat from dairy products and exclusive butter versus margarine/low fat spread use) and respiratory and atopic outcomes in 5-year-old children participating in the SEATON birth cohort study.

In **chapter 4**, we longitudinally investigate the associations between maternal food consumption during pregnancy and symptoms of asthma from 1 to 8 years of age in the PIAMA birth cohort. Previous prospective studies maternal diet during pregnancy and childhood asthma or allergy mostly used cross-sectional statistical analyses to investigate this relationship. To our knowledge, the study described in chapter 4 is the first to use longitudinal statistical techniques to investigate this relationship over a longer time period. Longitudinal analyses are important to reveal temporality of effects, and to strengthen the evidence of causal relationships between

maternal diet during pregnancy and childhood allergic disease.

Dietary exposure used in epidemiological studies investigating relations between nutrition and children's health, is usually just measured once in time. It is not yet clear how well dietary consumption measured at one point in time reflects dietary habits over a longer period. In **chapter 5** we assess the stability of dietary habits during childhood.

Evidence from epidemiological studies on the association between childhood diet and allergic disease is still inconsistent. Studies use different methodology and often relate dietary exposure at one point in time to health outcomes at the same point in time or a (few) year(s) later. Recently, the focus has shifted to the importance of diet during early life (from conception to 2 years of age). The use of longitudinal childhood dietary data could give insight in effects of long-term dietary exposure, and of differences in effects of dietary habits at early and at later age. In **chapter 6**, we investigate if symptoms of asthma or atopy at 8 years of age are associated with long-term dietary exposure from 2 to 8 years of age, and whether associations differ for consumption at early or later age.

This thesis concludes with a general discussion of the results presented in this thesis (**chapter 7**).

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Chapter 2

Foods, nutrients and allergic disease in children – review of the evidence

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2.1 Introduction

Diet is a relatively recently recognised potential risk factor for asthma. The number of epidemiological studies providing evidence on the possible association between diet and asthma or allergy, has increased over the last decades. Most of the available studies have reported on possible protective effects of long chain n-3 PUFAs in fish, dietary antioxidants such as vitamin C, vitamin E and selenium and the possible deleterious effects of n-6 PUFAs and sodium. This review focuses on the epidemiologic evidence from observational and intervention studies for an association between dietary intake and clinical indicators of asthma or allergy in children, although some evidence from studies in adults will be discussed as well.

2.2 Methods

We considered observational and intervention studies using Pubmed search on the terms 'diet' 'asthma' 'allergy' 'atopy' in combination with 'vitamin*', 'antioxidant', 'sodium', 'salt','magnesium', 'fruit', 'vegetables', or 'selenium', or 'flavon*' The key characteristics of observational and intervention studies in children that we used in this review are displayed in tables (study population and design, specification of dietary intake or intervention measure, clinical end-point for asthma or allergy, confounding variables, and a summary of results). To provide an overview of the available epidemiological evidence for each food or nutrient group there are separate tables for dietary electrolytes (sodium, potassium, magnesium) (table 2.1), lipids (including foods containing these lipids) (table 2.2), antioxidants (fruit and vegetables, vitamin C, vitamin E, β-carotene, vitamin A, and selenium) (tables 2.3–2.8) and zinc and copper (table 2.9). Furthermore a separate table for the evidence on maternal diet during pregnancy was created (table 2.10). The results of the studies are summarised by dietary factor as: 'no' association, 'beneficial' association or 'harmful' association. Strong study characteristics received more weight in the interpretation of the evidence. The tables include observational and intervention studies in which (i) dietary intake or biomarkers of intake were measured as the independent variable (excluding studies on nutrient status) and (ii) outcomes were measured that are clinically relevant for asthma or allergy such as atopy, lung function, symptoms, doctor's diagnosis. Other studies using biomarkers of nutrient intake as an end-point and studies on subclinical end-points of asthma or allergy such as effects on cytokines, are particularly useful to provide evidence for biological mechanisms but are not included in the epidemiological evaluation.

2.3 General considerations

The evidence from the epidemiological studies was weighted and interpreted taking some general considerations into account. Strength of the study design: The indicators for a causal effect become stronger going from ecologic, to cross-sectional, to longitudinal and finally to intervention studies. Although the number of prospective

observational and intervention studies has increased over the last few years, these are still a minority of the evidence. Strengths and weaknesses of the specific study designs can be found in the book of Rothman.[1]

Some studies have controlled for only a few nutritional or non-nutritional confounders. Although there may be good practical or statistical reasons for this, it makes the findings of such studies less robust than those from studies that adopted a more rigorous approach to control for confounding.

2.3.1 Temporal relationship

In the interpretation of the available studies, it is assumed that the dietary intake assessed at the time of the study is relevant to the end-points measured in the study. However, there are several complications that need further consideration. First, the induction time for the effect of dietary intake of foods or nutrients, as well as the duration of the effect is largely unknown. Secondly, the time window of exposure relevant for the development of asthma or allergy is likely to be during pregnancy or at very early age when the immune system is developing, whereas most of the available studies were performed at later ages. Thirdly, reverse causation have occurred in cross-sectional studies. On the one hand, the possible association between dietary intake and asthma or allergy is not widely known, except for the effects of foods on development of food allergy. Therefore it is unlikely that the general population would change their dietary pattern to prevent asthma or allergy. On the other hand, reverse causation may play a role when those with asthma or allergy avoid certain foods, and reversed biomarker assessment can play a role when, for instance oxidative stress associated with asthma depletes the level of antioxidants in blood. In longitudinal studies, the temporal relationship is clearer, but selective avoidance may occur e.g. when atopic parents anticipate the elevated risk of their children by altering their diet.

2.3.2 Nutrients versus foods

The effects of dietary intake on asthma or allergy may be caused by specific nutrients, by specific foods, or by aspects of a 'healthy diet' associated with intake of these foods or nutrients. Studying the role of individual nutrients is relevant to the understanding of the biological mechanism behind the observed associations. Also, consistent evidence from supplementation studies showing beneficial effects on clinical indicators of asthma or allergy is promising for prevention of asthma or allergy by nutrient supplementation in high-risk groups or asthma patients because dietary supplementation is often easier to implement than lifestyle changes. However, it is often observed that supplementation studies show effects on biological markers of nutrient intake and sometimes also on subclinical end-points of asthma or allergy while clinical effects are absent. An explanation may be that the effect observed in observational studies was not caused by the nutrients as such, but by other aspects associated with the intake of foods containing these nutrients, for example complex interactions between nutrients present in these foods or by a lifestyle associated with intake of these

foods (confounding). In that situation, preventive measures should be focused on the implementation of guidelines for a healthier diet or a healthy lifestyle rather than on the specific nutrient or food. So far, very few studies have analysed dietary patterns in relation to asthma or allergy.

2.3.3 Misclassification of dietary intake

When dietary assessment methods do not assess total dietary intake, misclassification of dietary intake is likely to occur. Some studies do not allow adjustment for total energy intake and do not give a complete estimate of nutrient intake. Few studies tend to address whether dietary reporting might have been biased, e.g. by identifying energy under-reporters and checking if results hold up when they are excluded. For nutrients whose assessment depends more on the intake of a specific food or food-group, misclassification is less of a problem. Studies in children are particularly liable to misclassification of dietary intake. Inherent to the large changes and variability in dietary habits of small children, there are no or few validated dietary assessment methods in children.

2.3.4 Multiple hypothesis testing and collinearity

As diet consists of so many different components which may be related to diet, alone or in combination, the assessment of different foods or nutrients simultaneously is important to avoid misclassification and to be able to attribute associations to specific foods or nutrients. However, careful consideration of all possible dietary factors automatically involves testing of multiple hypotheses. Also, the close correlation between dietary intake of certain foods or nutrients leads to collinearity of independent variables. Both aspects of the assessment of total dietary intake lead to difficulties in the statistical interpretation of results.

2.3.5 Definition of clinical end-points of asthma and allergy

A clinical definition of asthma is often lacking in epidemiological studies. Doctor's diagnosis of asthma is often self-reported and if it originates from medical records, there is no guarantee for standardisation of the clinical definition. Therefore, associations are often reported with symptoms of asthma or allergy, such as wheeze, shortness of breath or self-reported allergy and medication use. Increasingly, clinical indicators such as bronchial hyperreactivity, serum IgE, skin sensitivity, and reversibility of lung function after bronchodilator use have been used. Although the absence of clinical and standardised diagnosis of asthma and allergy limits the interpretation of the results of epidemiological studies for the overall conclusions, the information on separate clinical indicators provides more specific evidence on biological plausibility of associations.

2.4 Dietary electrolytes

2.4.1 Sodium and potassium

In 1987, Burney[2] noted that geographical variations in asthma mortality across England and Wales were accompanied by similar regional differences in table salt consumption for men and children, but not for women. High salt consumption was present in regions with high asthma mortality. Increasing dietary sodium could cause an increase in airway reactivity through potentiation of the electrogenic sodium pump in the membrane of the airway smooth muscle.[3] In the interpretation of the evidence we put most weight on epidemiologic studies with urinary sodium excretion as a measure of dietary intake.

Evidence from epidemiological studies in children are shown in table 2.1. The only observational study in children using urinary excretion as a measure of intake showed no association for sodium excretion and a harmful association for potassium excretion with airway reactivity and the respiratory symptoms cough, phlegm and wheeze in boys, but not in girls.[4] Salt intake estimated as table salt use,[4] supplemental salt use,[5] or regular salt use,[6] was harmfully associated with respiratory symptoms in boys,[4] or asthma.[5,6] The studies conducted in Kenya,[5] and Saudi-Arabia[7] may be more difficult to interpret. In tropical climates where sufficient salt intake is necessary for good health status, a low salt intake may reflect a poor diet and thus have a different association with asthma or allergy.

There have been several experimental studies on sodium in relation to asthma and allergy as well; however, most of them are conducted in adults. The first experimental study was performed in a small group of asthmatic patients and non-asthmatics.[8] This study showed an increased bronchial hyperreactivity to histamine in asthmatics after doubling salt intake during 1 month, whereas there was no change in non-asthmatics. Further studies (mostly cross-over trials) were restricted to asthmatic patients and showed some evidence that high sodium diets are associated with increased airway reactivity in men[9,10] and decreased lung function and increased medication use in men and women.[9–11] However, the study of Lieberman and colleagues[12] observed no effect of high or low salt intake on the severity of asthma. The study of Carey and collegues[10] was the only intervention study that also included children (table 2.1). A Cochrane review of intervention studies on salt reduction in asthmatics showed some evidence of an improvement in pulmonary function, but concluded that there was no sufficient evidence of a beneficial effect of salt reduction for the management of asthma.[13]

In conclusion, intervention studies provide substantial evidence for an adverse effect of a high sodium diet on airway reactivity and other respiratory end-points in asthmatic subjects. The harmful association is less clear in observational studies in the open population. This may be due to methodological problems of measuring salt intake adequately or presence of inadequate measurement of confounding variables. Intervention studies provide insufficient evidence for a beneficial effect of salt reduction in asthmatics, but some patients may benefit from restricting salt intake to prevent bronchoconstriction.

2.4.2 Magnesium

Magnesium may play a beneficial role in the prevention and treatment of asthma through relaxation of the bronchial smooth muscle, and inhibition of the release of acetylcholine from cholinergic nerve terminals and histamine from mast cells.[14]

Beneficial effects of magnesium intake on lung function and airway reactivity were observed in several cross-sectional studies in adults.[15–17] The findings by Dominguez and colleagues[18] and Emelyanov and colleagues[19] that asthma patients had lower levels of intracellular magnesium supports this observation, although magnesium status does not necessarily reflect dietary intake of magnesium due to metabolic regulation. Other studies found no association between magnesium intake[20,21] or magnesium deficiency[19,21] and asthma/asthma severity or lung function.

Nebulised inhaled magnesium sulfate in the treatment of an acute asthma exacerbation, appears to have benefits with respect to improved pulmonary function in patients with severe asthma.[22] However, few intervention studies have examined the effect of oral magnesium supplementation on other respiratory end-points. In adults, magnesium supplementation had a beneficial effect on asthma symptoms, whereas the only intervention study in children showed a beneficial effect on bronchodilator use[23] (table 2.1).

In conclusion, there are some indications for a beneficial effect of magnesium on indicators of asthma and allergy from observational and intervention studies although no associations were observed in more informative prospective studies. More observational or intervention studies using reliable methods for estimation of dietary magnesium intake and larger study populations are needed to confirm these conclusions.

Table 2.1: *Dietary electrolytes in relation to asthma and allergy in epidemiological studies in children*

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Carey et al.[10] 1993 UK	27 male asthma patients 12-68 yrs RCT	Low sodium diet + slow sodium capsules (200 mmol/day) vs low sodium diet + placebo; cross-over after 5 weeks	PEFR, daily symptom score, medication use, FEV_1, BHR	–	Salt intake: harmful association with BHR, medication use, PEFR and FEV_1
Pistelli et al.[4] 1993 Italy	2439 children 9-16 yrs Cross-sectional	- Table salt use - Sodium and potassium excretion (untimed sample)	Symptoms: cough, phlegm, wheeze; BHR (PD_{20})	Age, sex, SES, parental smoking, familial asthma, atopy	- Table salt use: harmful association with symptoms in boys only - Sodium excretion: no associations - Potassium excretion: harmful association with symptoms and BHR in boys only
Mohamed et al.[5] 1995 Kenya	77 asthmatic children, 77 controls 9-11 yrs Case-control	Supplemental daily salt intake: mean cases 817 mg vs controls 483 mg	Asthma	Damp damage in child's bedroom, furniture, rugs and carpets in child's bedroom, air pollution in the house	Extra daily salt intake: harmful association with asthma: Adj OR for 1 SD increase: 1.6 (1.1-2.4)
Demissie et al.[6] 1996 Canada	187 asthmatic children, 145 age and sex matched controls 5-13 yrs Case-control	Dietary salt intake (quartiles) FFQ	Asthma (defined as 10% decline in FEV_1 after exercise and/or history of dd-asthma); BHR	Age, sex, race, parental asthma, SES, pre- and postnatal exposure to tobacco smoke	Salt intake: harmful association with BHR but no association with asthma

Table 2.1 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Hijazi et al.[7] 2000 Saudi-Arabia	114 cases and 202 controls from population of 1444 children 12 yrs Case-control	- Sodium intake: mean (SD) cases 2313 (401) vs controls 2508 (406) mg/day - Potassium intake: mean (SD) cases 4581 (614) vs controls 4824 (711) mg/day - Sodium intake:(tertiles) FFQ	Asthma/wheeze in the last 12 mths	Place of residence, nationality, sex, mother's education, family history of asthma and wheeze and positive skin prick test	- Sodium: beneficial association with asthma/wheeze ($p<0.0001$) (uv) - Potassium: beneficial association with asthma/wheeze ($p=0.002$) (uv) - Sodium: beneficial association with asthma/wheeze: OR lowest vs highest tertile 2.88 (1.42–5.87) (mv)
Gilliland et al.[84] 2003 USA	Children's Health Study 2566 children 11-19 yrs Cross-sectional	Sodium, potassium and magnesium intake (quintiles) FFQ	Lung function: FVC, FEV_1, FEF_{25-75}, FEF_{75}	Cohort, community, spirometer, technician, barometric pressure, spirometer temperature, race, total energy intake	- Sodium: harmful association with FVC in boys only - Potassium: beneficial association with FVC and FEV_1 in girls - Magnesium: beneficial association with FVC in boys and FEF_{75} in girls
Bede et al.[23] 2003 Hungary	89 asthmatic children 4-16 yrs RCT	Mg supplementation (12 wks) with 200/290 mg/day Mg citrate vs placebo (260 mg/day glucose)	Bronchodilator use	-	Magnesium supplementation: beneficial association on bronchodilator use ($p<0.05$)
McKeever et al.[93] 2004 USA	NHANES III 4428 children 6-16 yrs Cross-sectional	Serum nutritional markers	Atopy: spt to common allergens	Age, sex, smoking, BMI, poverty index, race/ethnicity	- Serum sodium: no associations with atopy - Serum potassium: no associations with atopy

2.5 Lipids

Black and Sharpe[24] hypothesized that in industrialised countries the increase in the prevalence of atopic diseases has been preceded and then paralleled by a fall in the consumption of saturated fat (animal fat) and oily fish (containing long-chain n-3-polyunsaturated fatty acids (PUFAs)) and an increase in the intake of fats containing n-6-PUFAs (margarine and vegetable oils), resulting in a shift in the n-6/n-3 ratio of the diet. One of the proposed mechanisms by which increasing dietary intakes of n-6 PUFAs from margarine and vegetable oils and decreasing intakes of n-3-PUFAs from oily fish could promote asthma incidence is through the critical role of PUFAs as precursors of the eicosanoids.[24] The most common dietary PUFAs are linoleic acid and α-linolenic acid, which are the parent fatty acids for the n-6 and n-3 PUFA families, respectively. Biological mechanisms have been extensively reviewed by Calder and colleagues.[25]

2.5.1 Long-chain n-3-polyunsaturated fatty acids and fish

Fish, in particular oily fish, is one of the most important sources of dietary long-chain n-3-PUFAs. Fish intake is therefore often used as a proxy of long-chain n-3-PUFA intake. Nevertheless, there is the possibility of confounding by other nutrients present in fish and confounding by healthy diet or lifestyle that may be associated with fish consumption.

Evidence from epidemiological studies in children are shown in table 2.2. The results of observational studies in children are inconsistent. Five studies reported beneficial effects of fish consumption on asthma, persistent cough and wheeze, nighttime breathlessness and rhinitis[26–30] whereas two other studies reported no association between fish consumption and incidence of wheeze, shortness of breath and rhinitis.[31,32] The prospective study of Dunder and colleagues[33] observed no association between fish intake and atopy at baseline, but after 9 years the children who developed an atopic disease had consumed less fish at baseline. The studies of Chatzi and colleagues in Spain and Crete found beneficial associations between fish intake and atopy as well.[34,35] The inconsistency in results from the available studies cannot be easily attributed to weaknesses of specific studies. The studies showing a beneficial association were generally well designed and reasonably powered. None of the studies in children were energy adjusted due to lack of total energy intake data. Longitudinality is a strong characteristic of two out of three studies that observed no associations. Finally, two studies from Taiwan and Japan observed adverse associations between fish intake and the prevalence of asthma in children.[36,37] However, the results from the Taiwanese study[36] were obtained from a cross-sectional univariate analysis, while the adverse association between fish intake and asthma observed in Japan[37] was significant only in one subgroup (fish consumption of one to two times a week vs one to two time a month as reference group) and had not been adjusted for the potentially confounding factors associated with socioeconomic status. The birth cohort study of Dunlop and colleagues[38] found a harmful association of fish intake in the first year of life and eczema. Fish oils are preparations containing relatively high amounts of

long-chain n-3 PUFAs. Several studies report anti-inflammatory effects of fish oil in patients with asthma, such as decreased 4-series leukotriene production[39–41] and leucocyte chemotaxis.[39,41] A number of randomised double-blind placebo-controlled studies of fish oil in asthma have been reported. A Cochrane review[42] included nine of these studies published between 1988 and 2000. It was concluded that there was "no consistent effect on FEV_1, peak flow rate, asthma symptoms, asthma medication use or bronchial hyperreactivity". They conceded that one study in children showed improved peak flow and reduced asthma medication use.[42] A more recent report covering 26 studies (both randomized, placebo-controlled and others) concluded that "no definitive conclusion can yet be drawn regarding the efficacy of n-3-fatty acid supplementation as a treatment for asthma in children and adults".[43]

In addition to attempts to treat asthma with fish oil supplementation, some studies have examined the protective effect of early life exposure to long chain n-3 fatty acids (table 2.10). The Childhood Asthma Prevention Study (CAPS) intervention study including unborn children at high risk of developing asthma, found that increasing the intake of n-3 fatty acids and limiting the intake of n-6 fatty acids had a beneficial effect on wheeze at 18 months of age[44] and significantly reduced atopic cough at 3 years of age.[45] However, there was no effect on the other end-points measured such as eczema, serum IgE or doctor's diagnosis of asthma.[45] At 5-year follow-up there was still no difference in prevalence of asthma, wheezing, eczema or atopy between the dietary intervention groups.[46] In a randomized controlled trial in which atopic pregnant women received fish oil or placebo, infants born to mothers in the intervention group showed reductions in cytokine responses and less wheeze, cough and food allergy[47,48]. However, it should be noted that the children were too young to study a diagnosis of asthma reliably. Recently, new evidence from observational studies investigating effects of maternal diet during pregnancy (table 2.10) have shown beneficial associations of maternal (oily) fish consumption during pregnancy and asthma,[49,50] atopy,[51] eczema,[52,53] and wheeze.[54]

In conclusion, the available observational and intervention studies do not support that there is a beneficial effect of dietary fish oil or long-chain n-3-fatty acids intake in children on clinically observable end-points in asthma and allergy, although it is biologically plausible that long-chain n-3-fatty acid intake has a protective effect on inflammatory response and immune regulation. Recent results from observational studies of maternal diet during pregnancy are promising, but results from the Australian intervention studies are not very convincing with respect to allergic disease outcomes.

2.5.2 N-6 fatty acids and specific dairy products

The intake of n-6 fatty acids has been estimated through assessment of dietary intake (dietary record keeping, semiquantitative FFQs, 24-hour recall), plasma levels, or cellular levels (table 2.2). Adjustment for total energy intake was rarely possible in the reported studies, therefore estimates of associations with foods rich in particular fatty acids do not necessarily represent associations with those fatty acids, as other sources of these fatty acids may not have been measured adequately. This should be

taken into account in studies where the intake of milk, butter, margarine and other dairy products was used as a surrogate marker for the intake of n-6 fatty acids, *trans* fatty acids or monounsaturated fatty acids (MUFAs). The estimation of dietary fatty acid intake by spread usage is particularly misleading as the commercial margarine vary in their proportions of different fatty acids. For example, margarine contains up to 20 times more of the n-6 PUFA linoleic acid than butter does. For milk, it is not clear whether associations should be attributed to the n-6 PUFA content of milk products, to the vitamin A content, or even may be due to microbial agents contained in milk such as endotoxins or other factors characteristic of the farming environment.[55,56]

Dietary intake of margarine or foods with high levels of n-6 fatty acids

Most of the epidemiological studies in children reported harmful associations between the intake of n-6 fatty acids through spreads and cooking oils and indicators of asthma. Haby and collegues[57] reported a harmful association between intake of PUFAs through spreads and cooking oils and asthma. However, no distinction between n-6 and n-3 fatty acids was made in this study. Other studies found harmful associations (sometimes in subgroups only) between margarine consumption and allergic sensitisation and rhinitis in boys,[58] between margarine consumption and wheeze,[31] and between margarine consumption and hay fever, but not sensitisation, asthma or bronchial hyperreactivity.[59] In a cross-sectional analysis, Dunder and colleagues[33] reported that a higher intake of margarine was associated with atopic disease in children. Margarine intake during pregnancy was not associated with atopy at 5 years of age.[51]

Dietary intake of milk

The few studies reporting on potential effects of milk intake fairly consistently suggest a beneficial association. In children, a protective effect of the intake of milk was observed in a longitudinal study,[32] and two cross-sectional studies.[7,28]

Dietary intake of butter

Three studies reported a beneficial association between butter consumption and indicators of asthma and allergy.[32,33,59] One recent cross-sectional study found no association between butter consumption and any of the end-points,[28] while another cross-sectional study reported a harmful association of butter intake with shortness of breath and wheeze.[31] Butter intake during pregnancy was not associated with atopy at 5 years of age.[51]

Dietary intake of other types of fatty acids

Studies on MUFA intake and symptoms of asthma, rhinitis or sensitisation in adults are inconsistent.[16,60–63] There are not many studies on the effects of MUFA intake in children. Huang and Pan[64] reported a beneficial association between the intake of

MUFA and asthma in 13 to 17- year-old adolescents, whereas Murray and colleagues[65] did not find an association between MUFA intake and atopic wheeze.

Only one study looking at the effects of *trans* fatty acids on asthma in children has been published so far. In this ecological analysis of the ISAAC study a harmful effect of *trans* fatty acids, in particular originating from products containing hydrogenated fats such as biscuits, cakes, and chips, on asthma symptoms in children was reported.[66] Owing to severe limitations of the ecological study design it is not possible to draw conclusions on the potential effects of *trans* fatty acid on end-points of asthma or allergy. The study of Salam and colleagues found that fish stick (a possible source of *trans* fats) consumption increased the risk of asthma in children.[49] Dietary saturated fat intake has been reported to be positively associated with asthma in children.[64]

In conclusion, there is some evidence that the intake of butter and whole milk may be beneficially associated with indicators of asthma or allergy. However, it is not clear to what extent the beneficial effect of milk and butter should be attributed to the fatty acid composition of the milk or other factors.

2.5.3 Lipids and atopic dermatitis

Another mechanism by which PUFAs could play a role in atopic disease might be through their critical role in normal epidermal structure and function. It has been suggested that atopic dermatitis is caused by a reduced rate of the enzyme $\Delta 6$ desaturase which is responsible for converting linoleic acid to γ-linolenic acid (GLA) and α-linolenic acid to stearidonic acid.[67,68] Several studies of adipose tissue, plasma, umbilical cord, erythrocyte and leucocytes in children and adults reporting elevated concentrations of linoleic acid metabolites support this concept.[67–73] However, other studies reported conflicting results.[74–76] In a review published in 2000, Horrobin concluded that the balance of evidence indicates that very high doses of linoleic acid (10-60 g/day) or modest doses of GLA resulted in clinical improvement of atopic dermatitis symptoms, particularly in itching.[77] On the other hand, in a recent meta-analysis of placebo-controlled trials including 19 studies of GLA (with borage oil, evening primrose oil or blackcurrant seed oil) and 5 studies of fish oil, the authors concluded that "oral fatty acid supplementation is of no proven effectiveness for the treatment of atopic dermatitis". However, it is possible that PUFA supplementation could be of benefit in young children or patients with severe atopic dermatitis.[78] In a postnatal dietary supplementation of genetically susceptible infants for the first 6 months of life with borage oil (100 mg/day GLA), GLA failed to prevent the expression of atopy at one year, but it tended to alleviate the severity of atopic dermatitis. The infants who had the highest increase in plasma phospholipid GLA level developed the least severe atopic dermatitis at the age of 1 year.[79]

Table 2.2: *Intake of lipids/lipid containing foods in relation to asthma and allergy in epidemiological studies in children*

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Hodge et al.[26] 1996 Australia	468 children 8-11 yrs Case-control	Fish (fresh, oily, non-oily) intake (freq of intake) SFFQ	BHR, wheeze in the last 12 months, current asthma (BHR and wheeze)	Sex, ethnicity, country of birth, atopy, early respiratory infection, parental smoking	Oily fish: beneficial association with current asthma: OR high vs low intake 0.26 (0.09-0.72)
Von Mutius et al.[59] 1998 Germany	1,492 and 2,311 children 9-11 yrs Repeated cross-sectional	Consumption of 22 food items	Hay fever, sensitisation, asthma, BHR	Family history of atopic disease, SES, number of siblings, use of wood or coal for heating, pets	- Margarine: harmful association with hay fever; no association with sensitisation, asthma and BHR - Butter: beneficial association with hay fever and sensitisation; no association with BHR and asthma
Weiland et al.[66] 1999 Germany	ISAAC Children from 155 centres around the world 13-14 yrs Ecological	Fatty acid intake assessed by using representative market baskets per country	Symptoms of asthma, rhinitis and atopic eczema	GNP	Trans fatty acids: harmful association with prevalence of asthma symptoms, rhinitis and atopic eczema ($p<0.001$)
Hijazi et al.[7] 2000 Saudi-Arabia	114 cases and 202 controls from population of 1444 children 12 yrs Case-control	- Fish intake (often, sometimes, rarely, never) - PUFA intake (adjusted for total fat) (mean (SD) cases 20.7 (3.6) vs controls 20.3 (3.7) g/day) - Milk intake (mean (SD) cases 2.35 (1.30) vs controls 2.70 (1.28) nr of portions daily) FFQ	Asthma or wheeze in the last 12 months	Social class, place of residence, nationality, sex, mother's education, family history of asthma/allergy, positive skin test	- Fish: no association with asthma/wheeze ($p=0.073$) (uv) - PUFAs: no association with asthma/wheeze (uv) - Milk: beneficial association with asthma/wheeze: OR <2 vs ≥ 2 portions/day: 2.4 (1.2-4.8) (mv)

Table 2.2 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Bolte et al.[58] 2001 Germany	2348 children 5-14 yrs Cross-sectional	Margarine/butter use on bread (margarine only or margarine + butter vs butter only)	Asthma or asthma-like bronchitis, hay fever, rhinitis symptoms, sensitisation	Age group, residence, parental education, parental atopy, presence of siblings, BMI	Margarine: harmful association with allergic sensitisation and rhinitis in boys: OR 1.6 (1.1-2.2) and 1.8 (1.1-2.8) resp.
Dunder et al.[33] 2001 Finland	231 atopic cases and 231 non-atopic controls in 1980 154 atopic cases and 154 non-atopic controls in 1986 Repeated Case-control: children who developed disease were compared with those who had not	Margarine, butter, fish intake 48h recall Serum fatty acids	Allergic rhinitis, allergic dermatitis or asthma (separate and together atopic disease)	Age, sex, region, education of mother	In 1980: - Margarine: harmful association with atopic dermatitis and atopic disease - Butter: beneficial association with atopic dermatitis and atopic rhinitis - Fish: no associations Comparison of cases who developed atopic disease in 1986 or 1989: - Margarine: no associations (in 1989) - Butter: beneficial association with atopic disease (in 1986 and 1989) - Fish: beneficial association with atopic disease (in 1989)
Ellwood et al.[129] 2001	ISAAC I Children from 53 ISAAC countries 13-14 yrs Ecological	Fat and fatty acid intake (total and from foods in % TEC) National food intake data Food balance sheet and food supply data	Current wheeze, sleep disturbing wheeze, allergic rhinoconjunctivitis, atopic eczema	GNP	- MUFA and PUFA intake from vegetable oils and animals: no associations - MUFA intake from vegetables: beneficial association with current wheeze and eczema - PUFA intake from vegetables: beneficial association with current wheeze, rhinitis and eczema - Fat and saturated fat intake: no associations

Table 2.2 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Haby et al.[57] 2001 Australia	974 children 3-5 yrs Cross-sectional	PUFA intake (High: used on bread or toast and when roasting or frying; low: use of MUFAs or saturated fats; medium: use of mixture of fats) Questionnaire	Recent asthma (dd-asthma + current wheeze/cough + using medication) and atopy	Atopy, parental asthma, serious resp. infection in first 2 yrs, breast fed ever, nr of older siblings	- PUFAs: harmful association with recent asthma: OR high vs low intake 2.0 (1.2-3.6)
Huang et al.[36] 2001 Taiwan	NAHSIT 1166 adolescents 13-17 yrs Cross-sectional	Intake of protein-rich and fat-rich foods (quartiles) (uv) and (high >75% vs low ≤75% of intake) FFQ	Dd-asthma, allergic rhinitis	Urbanisation level	- Oily fish: harmful association with asthma (p=0.01) (uv) - Butcher's meats, liver and deep fried foods: harmful association with asthma: OR high vs low intake: 2.3 (1.1-4.8) and 2.1 (1.1-4.3) - Liver: harmful association with rhinitis: OR high vs low intake: 1.7 (1.1-2.6)
Huang and Pan.[64] 2001 Taiwan	NAHSIT 1166 adolescents 13-17 yrs Cross-sectional	Dietary fat intake (mean (SD)) 24-h recall	Asthma, rhinitis	Sex, urbanisation level	- Saturated fat: harmful association with asthma: OR for 1 SD increase: 2.0 (1.4-2.9) - MUFAs: beneficial association with asthma: OR for 1 SD increase: 0.7 (0.4-1.0)
Takemura et al.[37] 2002 Japan	1673 asthmatic children, 22109 controls 6-15 yrs Case-control	Fish intake (nr of times/week) FFQ	Asthma	Age, gender, parental history of asthma, vegetable and fruit intake	- Fish: harmful association with asthma: OR 1-2/week vs 1-2/month: 1.1 (1.0-1.2)

Table 2.2 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Antova et al.[27] 2003	CESAR 20271 children 7-11 yrs Cross-sectional	Fish intake (≤1/month vs <1/month) FFQ	Winter cough, persistent cough, wheeze ever, current wheeze	Age, sex area, pets, indoor moisture, gas cooking/heating, smoking, mothers education, fathers occupation, parental allergy, respondent, overcrowding, all other nutritional factors	- Fish: beneficial association with persistent cough, wheeze ever and current wheeze: OR low vs high intake 1.2 (1.0-1.3), 1.1 (1.0-1.3) and 1.2 (1.1-1.4) resp.
Farchi et al.[31] 2003 Italy	SIDRIA Baseline 5257 children, 1 yr follow-up 4104 children 6-7 yrs Cohort	- Fish (never vs 3-4/week), bread and butter (never vs >4/week) and bread and margarine intake (never vs 1-2/week) SFFQ	Wheeze, shortness of breath with wheeze, rhinitis in the past 12 months	Sex, study area, paternal education, household crowding, maternal/paternal smoking, dampness/mould, parental asthma	- Fish: no associations - Bread and butter: harmful association with shortness of breath with wheeze: OR never vs >4/week 3.1 (1.2-8.3) - Bread and margarine: harmful association with wheeze: OR never vs 1-2/week 2.5 (1.3-5.1)
Wijga et al.[32] 2003 Netherlands	PIAMA Baseline 4146 children, 3 yr follow-up 2978 children 2-3 yrs Birth cohort	Fish (≤1/week vs >1/week) and dairy product intake (≤1/week vs 6-7/week) (at 2 yrs) FFQ	Ever asthma, recent asthma, recent wheeze (at 3 yrs)	Sex, birth weight, presence of older siblings, parental allergy, maternal level of education, breast feeding for at least 8 wks, smoking in the home and during pregnancy, region, parental asthma	- Fish: no associations (uv) - Full cream milk, milk products and butter: beneficial association with asthma end-points and/or recent wheeze
Andreasyan et al.[130] 2005 Australia	THIS/CARHS 499 children mean 8.71 yrs Cross-sectional	Fish intake (never vs ≤1/week or >1/week) SFFQ	Atopy: spt to ryegrass, hdm and other aeroallergens, asthma and hay fever	Sheepskin or plastic mattress use in infancy, sex, number of siblings	Fish: beneficial association with ryegrass-pure sensitisation: OR never vs ≤1/week 0.37 (0.15-0.90); no association with hdm-pure sensitisation: OR never vs ≤1/week 0.87 (0.36-2.13)

Table 2.2 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Kim et al.[28] 2005 Sweden	1014 children 5-14 yrs Cross-sectional	- Meat, fish, fresh milk, fermented milk and fast food intake (code 0-4 (where 0=never and 4=daily)) - Consumption of butter, margarine, olive oil, rape seed oil, and poly-unsaturated oils (yes/no) Questionnaire	Wheeze, daytime breathlessness, nighttime breathlessness, dd-asthma, current asthma, atopic sensitisation (self-reported)	Age, gender, and all other dietary factors at the same time	- Meat: beneficial association with dd-asthma: OR per 1 unit increase 0.64 (0.41-0.99) - Fish: beneficial association with nighttime breathlessness, dd-asthma and current asthma: OR per 1 unit increase 0.36 (0.17-0.78), 0.54 (0.35-0.84) and 0.51 (0.31-0.84) resp. - Fresh milk: beneficial association with dd-asthma and current asthma: OR per 1 unit increase 0.75 (0.61-0.94) and 0.77 (0.59-0.99) resp. - Butter: no associations - Margarine: beneficial association with wheeze: OR 0.42 (0.21-0.85) - Olive oil: beneficial association with dd-asthma: OR 0.47 (0.25-0.91) - Poly-unsaturated oil: harmful association with wheeze: OR 1.91 (1.14-3.19)
Dunlop et al.[38] 2006 Slovakia	1990 children follow-up 1 yr 1yr Birth cohort	Fish and milk intake in 1st yr of life Questionnaire	Infantile atopic eczema	Family history of atopy, parental education, pregnancy and fetal factors, region, residential factors, fuel type for heating/cooking, parental smoking, other household exposures, pets/livestock, breastfeeding, introduction of solids	- Fish: harmful association with eczema: OR 1.56 (1.1-2.3) - Milk: no association with eczema

Table 2.2 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Murray et al.[65] 2006 UK	37 sensitised children with recurrent wheeze, 37 controls 3-5 yrs Nested case-control	PUFA, MUFA and saturated fat intake (g/day) SFFQ	Atopic wheeze	Cases and controls were matched for age, sex, parental atopy, indoor allergen exposure and pet ownership, analyses were adjusted for total fat intake	- PUFAs: harmful association with sensitisation and wheeze: GM (95% CI) 7.0 (6.4-7.6) vs 5.7 (5.2-6.2) g/day - MUFAs: no association - Saturated fat: no association
Tabak et al.[29] 2006 Netherlands	ISAAC II 598 children 8-13 yrs Cross-sectional	Fish and dairy product intake (tertiles) SFFQ	Current wheeze, current asthma, atopy, BHR	Maternal educational level, foreign descent, total energy intake	- Fish: beneficial association with current wheeze and current asthma: OR lowest vs highest tertile 0.42 (0.20-0.89) and0.32 (0.13-0.81) resp. - Dairy products: no associations
Chatzi et al.[34] 2007 Spain	460 children 6.5 yrs Cross-sectional	Fish intake (tertiles) SFFQ	Current wheeze, atopy (spt), atopic wheeze	Gender, maternal asthma or atopy, total energy intake, fish intake during pregnancy, BMI	- Fish: beneficial association with atopy: OR higest vs lowest tertile 0.43 (0.21-0.90) (p-trend<0.05)
Chatzi et al.[35] 2007 Greece (Crete)	690 children 7-18 yrs Cross-sectional	Fish ($\geq$2/week), dairy product (1/daily), margarine (>1/week) Mediterrenean diet score	Wheezing ever, current wheezing, allergic rhinitis ever, current allergic rhinitis, atopy (spt)	Age, sex, BMI, parental asthma, nr of older siblings	- Fish: beneficial association with atopy OR 0.70 (0.47-1.03) - Dairy products: no associations - Margarine: harmful associations with current wheeze, rhinitis ever, current rhinitis: OR 2.19 (1.01-4.82), 1.99 (1.32-3.00) and 2.10 (1.31-3.37) resp.

Table 2.2 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Garcia-Marcos et al.[30] 2007 Spain	ISAAC III 20106 children 6-7 yrs Cross-sectional	Seafood, butter, margarine and milk intake (never/occasional vs 1-2/week or ≥3/week) Questionnaire	Current occasional asthma, current severe asthma, rhinitis	Sex, obesity, maternal smoking, siblings, exercise	- Seafood: beneficial association with severe asthma and rhinitis: OR ≥3/week vs never/occasional 0.53 (0.35-0.80) and 0.67 (0.53-0.85) resp. (p–trend<0.001) - Butter: no associations - Margarine: no associations - Milk: beneficial association with severe asthma and rhinitis: OR ≥3/week vs never/occasional 0.50 (0.28-0.90) and 0.68 (0.47-0.97) resp. (p–trend<0.001)

2.6 Antioxidants

Dietary antioxidants may protect the respiratory system against cell damage from oxidative stress. The most important antioxidant nutrients are vitamin C, vitamin E, β-carotene, vitamin A and selenium. Fruit and vegetables are important sources of antioxidant nutrients. These antioxidants may each play different roles in protecting lung tissue against damage. Some antioxidants could also exert their effects on asthma and atopic diseases via non-antioxidant properties (reviewed in Devereux and Seaton[80]). Vitamin E, selenium and quercetin have been reported to influence Th-cell differentiation towards Th1 immunophenotype. Reduced antioxidant status during fetal and early life would then increase the likelihood of a failure to switch from the Th2 to the Th1 immunophenotype in the neonate. If antioxidants exert their beneficial influences early in life, antioxidant strategies would only be successful if targeted towards mothers during pregnancy and very young children (0-5 years). A number of studies have investigated the associations between maternal antioxidant or antioxidant-rich food intake during pregnancy and atopic diseases. See table 2.10.

2.6.1 Fruit and vegetables

Most of the observational (prospective and cross-sectional studies in children) show beneficial effects of fruit, fruit juice, and/or vegetable intake on respiratory symptoms like wheeze, cough and shortness of breath[7,27,31,81,82] and pulmonary function.[83,84] One study reported a harmful association of fruit intake with asthma and of vegetable intake with sensitisation.[28] Children included in these studies were mostly between 5 and 12 years of age. A birth cohort study in preschool children[32] and a population-based cross-sectional survey in teenagers[64] found no association of fruit, fruit juice and vegetable intake with prevalence of asthma or wheeze (table 2.3).

In conclusion, there is relatively consistent evidence from observational studies for a beneficial effect of fruit and vegetables on pulmonary function than for other indicators of asthma or allergy. It is not clear whether beneficial effects of fruit and vegetable intake should be attributed to specific nutrients (antioxidants, flavonoids etc.) or whether these merely reflect other aspects of healthy lifestyle.

2.6.2 Flavonoids and flavonoid-rich foods

Flavonoids are polyphenolic compounds that are categorized according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, antocyanidins and chalcones.[85] Flavonoids are powerful antioxidants capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals. Foods (e.g. apples, onions, and soy) and beverages (e.g. tea, red wine) rich in flavonoids have been associated with a decreased risk of several diseases (cardiovascular diseases, cancer, COPD and asthma).[86] Dietary intake of flavonoids is usually assessed by food composition tables which are applied to estimates of food intake by questionnaires.

Several studies have looked at the relation between flavonoid rich foods and drinks (apples, onions, tea, red wine) asthma and/or allergy in adults. Shaheen and

colleagues,[87] Knekt and colleagues,[88] and Woods and colleagues,[89] found beneficial associations between apple consumption and lung function. Tabak and colleagues[90] found that solid fruit, but not tea, intake was beneficially associated with COPD. Knekt and colleagues[88] found a beneficial association between total flavonoid intake and the incidence of asthma. Separate analyses for specific flavonoids showed significant beneficial associations between the intake of quercetin, hesperetin, and naringenin and the incidence of asthma. Tabak and collegues[90] found beneficial associations between total flavonoid intake and lung function and COPD symptoms (cough, phlegm and breathlessness). The study of Okoko and colleagues found a beneficial association between children apple juice (but not apples) intake and wheeze,[91] and results of the SEATON study reported in this thesis found an association of maternal apple consumption during pregnancy and wheeze and asthma in 5-year-old children.[92] In conclusion, there are some indications that the intake of flavonoids or flavonoid rich foods (especially apples) has a beneficial effect on asthma and/or COPD in adults. Evidence from studies in children is still very limited, but as yet there is no evidence for harmful effects.

Table 2.3: *Fruit, vegetable and whole grain product intake in relation to indicators of asthma and allergy in observational studies in children*

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Cook et al.[83] 1997	278 children 8-11 yrs Cross-sectional	Fresh fruit (summer+winter), green salad (summer + winter) and green vegetable intake: (never vs >1/day) FFQ	FEV_1, wheeze	Age, sex, height, town, instrument, observer, room temperature, BMI, social class, passive smoking	- Fruit: beneficial association with FEV_1: 79 (22-136) ml higher FEV_1 for high vs low intake; no association with wheeze - Vegetables and salad: beneficial association, with FEV_1 (less than fruit); no association with wheeze
Forastiere et al.[813] 2000 Italy	SIDRIA 18737 children 6-7 yrs Cross-sectional	Citrus fruit and kiwi fruit intake (winter) (<1/week vs 5-7/week) FFQ	Wheeze symptoms, cough symptoms and rhinitis	Sex, study area, paternal education, household density, maternal/paternal smoking, dampness/mould, parental asthma	Citrus/kiwi fruit: beneficial association with various wheeze symptoms, nocturnal cough, chronic cough and rhinitis
Hijazi et al.[7] 2000 Saudi-Arabia	114 cases and 202 controls from a population of 1444 children 12 yrs Case-control	Fruit and vegetable intake: mean (SD) daily number of portions FFQ	Asthma or wheeze in the last 12 months	–	- Fruit: no association with asthma/wheeze (uv) - Vegetables: beneficial association with asthma/wheeze (p=0.007) (uv)
Ellwood et al.[129] 2001	ISAAC I Children from 53 countries 13-14yrs Ecological	Vegetable intake in g/day or % TEC; ranged from 18-470 g/day and 0.3-3% TEC National food intake data, food balance sheet and food supply data	Current wheeze, sleep disturbing wheeze, allergic rhinoconjunctivitis, atopic eczema	GNP	- Vegetables (g/day): beneficial association with current wheeze and eczema: 2% (p=0.041) and 2% (p=0.001) decrease in disease resp. for 100 g increase in consumption - Vegetables (% TEC): beneficial association with current wheeze, rhinitis and eczema: 31% (p=0.035), 27% (p=0.051) and 23% (p=0.002) decrease in disease resp. for an increase of 10% TEC

Table 2.3 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Huang et al.[36] 2001 Taiwan	NAHSIT 1166 adolescents 13-17 yrs Cross-sectional	Vegetable, fruit and juice intake (quartiles) FFQ	Dd-asthma, allergic rhinitis	–	- Vegetable, fruit and juices: no association with asthma - Fruits and juices: harmful association with rhinitis (p=0.039)
Antova et al.[27] 2003	CESAR 20271 children 7-11 yrs Cross-sectional	Fruit and vegetable intake (summer + winter) (<2/week vs >4/week) FFQ	Winter cough, persistent cough, wheeze ever, current wheeze	Age, sex area, pets, indoor moisture, gas cooking/heating, smoking, mothers education, fathers occupation, parental allergy, respondent, overcrowding, all other nutritional factors	- Fruit (summer): beneficial association with winter cough and persistent cough: OR <2/week vs >4/week 1.40 (1.10-1.79) and 1.35 (1.01-1.82) resp. - Fruit (winter): beneficial association with winter cough: OR 1.28 (1.09-1.51) - Vegetables: inconsistent associations with cough symptoms
Farchi et al.[31] 2003 Italy	SIDRIA Baseline of 5257 children, 1 yr follow-up of 4104 children 6-7 yrs Cohort	Cooked vegetable, summer tomato, fresh fruit and citrus fruit intake (never vs >4/week) SFFQ	Wheeze, shortness of breath, rhinitis and cough symptoms in the past 12 months	Sex study area, paternal education, household crowding, maternal/paternal smoking, dampness/mould, parental asthma	- Cooked vegetables: beneficial association with nocturnal and chronic cough - Summer tomatoes: beneficial association with shortness of breath, nocturnal and chronic cough - Fresh fruit: beneficial association with shortness of breath, nocturnal cough and chronic cough - Citrus fruit: beneficial association with nocturnal cough; (All sig p-values for trend)

Table 2.3 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Gilliland et al.[84] 2003 US	Children's Health Study 2566 children 11-19 yrs Cross-sectional	Fruit and vegetable intake (quintiles) Fruit juice intake (no intake vs some intake) FFQ	Pulmonary function: FVC, FEV_1, FEF_{25-75}, FEV_1/FVC ratio	Community, grade, spirometer, technician, pressure, log (height), age, race, asthma, respiratory illness at lf test, in utero exposure to maternal smoke, current ETS exposure, total energy intake	- Fruit and vegetables: no associations - Fruit juices: beneficial associations with FVC and FEV_1 in boys
Wijga et al.[32] 2003 Netherlands	PIAMA Baseline 4146 children, 3 yr follow-up 2978 children 2-3 yrs Birth cohort	Fruit, fruit juice and vegetable intake ($\leq$ 5/week vs 6-7/week) (2yrs) FFQ	Ever asthma, recent asthma, recent wheeze (3yrs)	-	Fruit, fruit juice and vegetables: no associations with asthma or wheeze (uv)
Wong et al.[82] 2004	10902 children 10 yrs Cross-sectional	Fruit and raw vegetable intake (frequency of intake) FFQ	Wheeze in the last 12 months	Propensity score, sex	- Fruit: beneficial association with current wheeze: OR $\leq$ 1/day vs >1/day 0.70 (0.54-0.89) - Raw vegetables: no association with current wheeze: OR $\leq$ 1/week vs <1/week 0.81 (0.64-1.03)
Kim et al.[28] 2005 Sweden	1014 children 5-14yrs Cross-sectional	Fruit and vegetable intake: code 0-4 (where 0=never and 4=daily) Questionnaire	Wheeze, daytime breathlessness, nighttime breathlessness, dd-asthma, current asthma, atopic sensitisation (self-reported)	Age, gender, and all other dietary factors at the same time	- Fruit: harmful association with dd-asthma and current asthma: OR per 1 unit increase 1.61 (1.12-2.32) and 1.52 (1.00-2.30) resp. - Vegetable: harmful association with atopic sensitisation: OR per 1 unit increase 1.30 (1.01-1.67)
Nja et al.[131] 2005 Norway	1994 children 6-16 yrs Cross-sectional	Fresh fruit or vegetable intake during the first yr of life (freq of intake) SFFQ	Asthma, sensitisation (spt)	Age, gender, area, parental atopy, mother's and father's education	Fruit or vegetables in 1st year: beneficial association with asthma: OR $\geq$1/day vs <1/day 0.57 (0.37-0.88)

Table 2.3 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Dunlop et al.[38] 2006 Slovakia	1990 children follow-up 1 yr 1yr Birth cohort	Citrus fruit intake in 1st yr of life Questionnaire	Infantile atopic eczema	Family history of atopy, parental education, pregnancy and fetal factors, region, residential factors, fuel type for heating/cooking, parental smoking, other household exposures, pets/livestock, breastfeeding, introduction of solids	Citrus fruit: no association with eczema
Pastorino et al.[132] 2006 Brazil	ISAAC III 528 adolescents (141 asthmatics. 387 control subjects) Case-control	Cooked vegetable intake Questionnaire	Asthma	-	Cooked vegetables: beneficial association with asthma: OR 0.37 (0.18-0.79)
Tabak et al.[29] 2006 Netherlands	ISAAC II 598 children 8-13 yrs Cross-sectional	Fruit, vegetable and whole grain product intake (tertiles) SFFQ	Current wheeze, current asthma, atopy, BHR	Maternal educational level, foreign descent, total energy intake	- Fruit: no association with wheeze, asthma, atopy or BHR - Vegetables: no association with wheeze, asthma, atopy or BHR - Whole grain products: beneficial association with asthma OR highest vs lowest tertile 0.43 (0.18-1.02)
Chatzi et al.[34] 2007 Spain	460 children 6.5 yrs Cross-sectional	Fruit, vegetable, fruity vegetable (tomatoes, eggplant, cucumber, green beans, zucchini) intake (tertiles) SFFQ	Current wheeze, atopy (spt), atopic wheeze	Gender, maternal asthma or atopy, total energy intake, fish intake during pregnancy, BMI	- Fruit: no associations - Vegetables: no associations - Fruity vegetables: beneficial association with wheeze and atopic wheeze: OR highest vs lowest tertile 0.38 (0.15-0.95) and 0.19 (0.04-0.95) resp. (p-trend<0.05)

Table 2.3 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Chatzi et al.[35] 2007 Greece (Crete)	690 children 7-18 yrs Cross-sectional	Fruit and vegetable intake (freq of intake) FFQ and Mediterrenean diet score	Wheezing ever, atopic wheeze (spt), atopy without wheeze, allergic rhinitis ever, current allergic rhinitis, seasonal allergic rhinitis	Age, sex, BMI, parental asthma, nr of older siblings	- Oranges: beneficial association with wheezing ever and allergic rhinitis ever: OR $\geq$1/day vs never 0.30 (0.11-0.90) and 0.29 (0.11-0.80) resp. - Kiwi fruit: beneficial association with allergic rhinitis ever: OR $\geq$1/day vs never 0.37 (0.16-0.86) (p-trend<0.05) - Grapes: beneficial association with ever wheeze and ever, current and seasonal allergic rhinitis: OR $\geq$1/day vs never 0.31 (0.14-0.64), 0.36 (0.16-0.81), 0.28 (0.10-0.80) and 0.18 (0.06-0.54) resp. - Fresh tomatoes: beneficial association with ever wheeze: OR $\geq$1/day vs never 0.32 (0.15-0.67) (p-trend<0.05) - Fruit, fruitjuice or vegetables (daily): no associations
Garcia-Marcos et al.[30] 2007 Spain	ISAAC III 20106 children 6-7 yrs Cross-sectional	Fruit and vegetable intake (never/occasional vs 1-2/week vs $\geq$3/week) Questionnaire	Current occasional asthma and current severe asthma	Sex, obesity, maternal smoking, siblings, exercise	- Fruit: beneficial association with rhinitis: OR $\geq$3/week vs never/occasional 0.71 (0.57-0.88) (p–trend<0.001) - Vegetables: no associations

Table 2.3 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Okoko et al.[91] 2007 UK	2640 children 5-10 yrs Cross-sectional	Apple, apple juice, pear, banana and miscellaneous fruit (soft, stoned, citrus, tinned and tropical fruit) intake fruit frquency questionnaire	Current wheeze, ever wheeze, ever asthma, exercise related wheeze, sleep disturbance due to wheeze and nocturnal cough	Sex, age group, paracetamol use, ibuprofen use, supplement use, ever lived on farm, mould or mildew in the house, finance source for home repairs, current and ever exposure to ETS, ethnic group, birth weight, breastfeeding, nr of parents living with child, nr of other children in home, mother's and father's educational level	- Apples: no associations - Apple juice: beneficial association with current wheeze: OR $\geq$1/day vs <1/month 0.53 (0.34-0.83) - Bananas: beneficial association with current wheeze and ever wheeze: OR $\geq$1/day vs <1/month 0.66 (0.44-1.00) and 0.69 (0.50-0.95) resp.

2.6.3 Vitamin C

Vitamin C (ascorbic acid) is water-soluble and maintains the antioxidant capacity in the intra- and extracellular aqueous phase of the cell (cytoplasm). Vitamin C scavenges the superoxide radical O2–, affects arachidonic acid metabolites, regenerates oxidized vitamin E, and may also play a role in immune function if present in neutrophils and lymphocytes. Vitamin C is mainly found in fruit and vegetables such as citrus fruits, tomatoes and broccoli.

Evidence of vitamin C intake is shown in table 2.4. Beneficial effects of vitamin C intake were observed on asthma/wheeze and lung function in two large observational studies in children.[64,84] Other observational studies reported no association of vitamin C intake with asthma, wheeze or atopic sensitization.[7,65,93,94] Serum vitamin C concentrations seemed to be beneficially associated with asthma in children,[95,96] but not with wheeze or lung function.[83] A prospective birth cohort study showed that vitamin C intake during pregnancy may have a harmful effect on the incidence of wheeze and eczema in the 2nd year of life.[97] However, this association disappeared at 5 years of age.[98]

Several experimental studies have evaluated whether antioxidant (pro)-vitamins were able to modulate the acute harmful effects of oxidative air pollution (ozone, nitrogen dioxide or a mixture of urban air pollution) on the lungs.[99–105] These studies differed in several design aspects and in outcome. However, the results of these studies indicate that antioxidant supplementation has the potential to protect against the acute damage of oxidative air pollution.[106] A Cochrane review of vitamin C intervention studies[107] concluded that evidence from randomised-controlled trials is insufficient to recommend a specific role for vitamin C in the treatment of asthma.

In conclusion, there are no clear indications from intervention studies in adults and children of a beneficial effect of vitamin C supplementation in asthma management, although there is some evidence that vitamin C supplementation protects against acute damage of oxidative air pollution.

Table 2.4: *Vitamin C in relation to end-points of asthma and allergy in epidemiological studies in children*

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Cook et al.[83] 1997	2650 children 8-11 yrs Nested case-control in cross-sectional study	Plasma vitamin C: Mean cases 64.1 vs controls 60.1 mol/l	Wheeze	-	Plasma vitamin C: no association with wheeze
Hijazi et al.[7] 2000 Saudi-Arabia	114 cases and 202 controls from a population of 1444 children 12 yrs Case-control	Vitamin C intake from diet (mean (SD) cases 139 (66) vs controls 150 (67) mg/day) and (tertiles) FFQ	Asthma or wheeze in the last 12 months	Sex, social class, place of residence, family history of asthma or hay fever, positive spt	Vitamin C: no association with asthma or wheeze (UV and MV)
Huang and Pan[64] 2001 Taiwan	NAHSIT 1166 adolescents 13-17 yrs Cross-sectional	Vitamin C intake (quartiles) 24-h recall	Dd-asthma	Urbanization, sex	Vitamin C: beneficial association with asthma (p-trend=0.004) (uv); OR lowest quartile vs higher intake 1.81 (0.88-3.71) (p=0.10) (mv)
Gilliland et al.[84] 2003 US	Children's Health Study 2566 children 11-19 yrs Cross-sectional	Vitamin C intake from diet (mg/day) or from diet + supplements (lowest decile vs higher intake) FFQ	Pulmonary function: FVC, FEV_1, FEF_{25-75}, FEV_1/FVC ratio	Community, grade, spirometer, technician, pressure, log (height), age, race, asthma, respiratory illness at lf test, in utero exposure to maternal smoke, current ETS exposure, total energy intake	Total vitamin C: beneficial association with FVC in boys; beneficial association with FEV_1 and FEF_{25-75} in girls
Harik-Khan et al.[95] 2004 US	NHANES III 4093 children 6-17 yrs Cross-sectional	Serum vitamin C level (mg/dl)	Dd-asthma	Age, gender, household size, BMI, household head gender, educational and employment status, parental asthma or hay fever, race, ETS in home, levels of serum antioxidant vitamins	Serum vitamin C: beneficial association with asthma: OR per mg/dl increase 0.72 (0.546-0.949)

Table 2.4 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
McKeever et al.[93] 2004 US	NHANES III 4428 children 6-16 yrs Cross-sectional	Serum vitamin C (mean (SD) 1.0 (0.4) mg/dl)	Skin sensitisation to allergens	Age, sex, smoking, BMI, poverty index, race/ethnicity	Serum vitamin C: no association with sensitisation
Romieu et al.[94] 2004 US	NHANES III 7851 children 2-16 yrs Cross-sectional	Vitamin C intake: (<60 mg/day vs ≥60 mg/day) 24-hr recall + questionnaire	Self-reported asthma or wheezing	BMI, gender, race, parental asthma and hay fever, passive smoking, poverty level, presence of atopy, number of hours spent watching tv, total caloric intake	Vitamin C intake: no association with asthma or wheeze
Rubin et al.[96] 2004 US	NHANES III 6153 children 4-16 yrs Cross-sectional	Serum vitamin C (mean (SD) 0.96 (0.43) mg/dl)	Prevalent asthma	Age sex, race, BMI, education level of head of household, household crowding, urban residence, passive and active smoking, parental history of asthma and/or hay fever, child history of hay fever, child avoidance of pets due to allergies	Serum vitamin C: beneficial association with asthma: OR for 1 SD increase: 0.81 (0.7-0.9)
Murray et al.[65] 2006 UK	37 sensitised children with recurrent wheeze, 37 controls 3-5 yrs Nested case-control	Vitamin C intake (GM (95% CI) cases 121.0 (104.2-140.6) vs 116.3 (96.0-140.7) mg/day) SFFQ	Atopic wheeze	Cases and controls were matched for age, sex, parental atopy, indoor allergen exposure and pet ownership, analyses were adjusted for total fat intake	Vitamin C: no association

2.6.4 Vitamin E

Vitamin E is a lipid-soluble antioxidant and a scavenger of free radicals in the lipid phase (cell membranes). It may protect the lungs against oxidant damage by breaking the lipid peroxidation chain reaction and converts oxygen radicals and lipid peroxyl radicals to less reactive forms. Vitamin E also has non-antioxidant effects on immune function.[108] Vitamin E is present in vegetable oils, margarine, butter, eggs, and green leafy vegetables.

Beneficial effects of vitamin E intake were reported with asthma[7] and lung function[84] in children, although Huang and Pan[64] found no effects of vitamin E intake on asthma. No associations between serum vitamin E levels and asthma or atopy was observed in several studies.[93,95,96] In a prospective birth cohort study, beneficial effects were observed for maternal vitamin E intake during pregnancy (but not serum levels) and cord blood mononuclear cell responses at birth,[109] wheezing in the second year of life[97] and wheeze, asthma, ventilatory function and exhaled nitric oxide at the age of 5 years.[98] Litonjua and colleagues also reported a beneficial effect of maternal vitamin E intake during pregnancy on wheeze in the first 2 years of life[110] (table 2.10). There are no studies on vitamin E supplementation in children.

There are no clear indications of a beneficial effect of childhood vitamin E intake on end-points of asthma or allergy. The observations from prospective birth cohort studies provide indications for a beneficial effect of maternal vitamin E intake during pregnancy on the incidence of wheeze and asthma in young children.

2.6.5 β-carotene

β-carotene (a precursor of vitamin A) is a lipid soluble antioxidant present in tissue membranes. It scavenges superoxide anion and reacts directly with peroxyl-free radicals. Carotenoids are present in brightly coloured fruit and dark green leafy vegetables.

No associations have been observed between serum β-carotene level and asthma[95,96] or skin sensitisation in children.[93] Murray and colleagues did not find an association between carotene intake and atopic wheeze in children.[65] However, Harik-Khan and colleagues[95] found beneficial association between α-carotene and asthma (table 2.6).

In conclusion the evidence for a beneficial effect of β-carotene on end-points of asthma or allergy in children is very limited.

Table 2.5: *Vitamin E in relation to end-points of asthma and allergy in epidemiological studies in children*

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Hijazi et al.[7] 2000 Saudi-Arabia	114 cases and 202 controls from a population of 1444 children 12 yrs Case-control	Vitamin E intake from diet (mean (SD) cases 7.16 (1.56) vs controls 7.71 (1.72) mg/day) and (tertiles) FFQ	Asthma or wheeze in the last 12 months	Sex, social class, place of residence, family history asthma/hay fever, positive spt	Vitamin E: beneficial association with asthma/wheeze (p=0.005) (uv); beneficial association with asthma: OR low vs high intake 3.00 (1.38-6.50) (mv)
Huang and Pan.[64] 2001 Taiwan	NAHSIT 1166 adolescents 13-17 yrs Cross-sectional	Vitamin E intake (quartiles) 24-h recall	Dd-asthma	-	Vitamin E: no association with asthma (p-trend=0.95) (uv)
Gilliland et al.[84] 2003 US	Children's Health Study 2566 children 11-19 yrs Cross-sectional	Vitamin E intake from diet (mg/day) or diet + supplements (lowest decile vs higher intake) FFQ	Pulmonary function: FVC, FEV_1, FEF_{25-75}, FEV_1/FVC ratio, PEF	Community, grade, spirometer, technician, pressure, log(height), age, race, asthma, respiratory illness at lf test, in utero exposure to maternal smoke, current ETS exposure, total energy intake	Vitamin E: beneficial association with FEF_{25-75}, FEV_1/FVC ratio and PEF in boys; no significant associations in girls
Harik-Khan et al.[95] 2004 US	NHANES III 4093 children 6-17 yrs Cross-sectional	Serum vitamin E level (mg/dl)	Dd-asthma	Age, gender household size, BMI, household head gender, educational, and employment status, paretal asthma or hay fever, race, smoker in the household, levels of serum antioxidant vitamins	Serum vitamin E: no association with asthma: OR 1.00 (0.999-1.001)
McKeever et al.[93] 2004 US	NHANES III 4428 children 6-16 yrs Cross-sectional	Serum vitamin E (mean (SD) 768.7 (173.1) μg/dl)	Skin sensitisation to allergens	Age, sex, smoking, BMI, poverty index, race/ethnicity, serum levels of total cholesterol and triglycerides	Serum vitamin E: no association with sensitisation: OR for 1 SD difference 1.01 (0.93-1.09)

Table 2.5 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Rubin et al.[96] 2004 US	NHANES III 6153 children 4-16 yrs Cross-sectional	Serum vitamin E (mean (SD) 775.9 (176.8) μg/dl)	Prevalent asthma	Age, sex, race, BMI, education level of the head of the household, household crowding, urban residence, passive and active smoking, parental history of asthma and/or hay fever, child history of hay fever, child avoidance of pets due to allergies, serum cholesterol, serum triglycerides	Serum vitamin E: no association with asthma: OR for 1 SD increase 0.95 (0.8-1.1)
Murray et al.[65] 2006 UK	37 sensitised children with recurrent wheeze, 37 controls 3-5 yrs Nested case-control	Vitamin E intake (GM (95% CI) cases 5.7 (5.0-6.6) vs 4.9 (4.4-5.4) mg/day) SFFQ	Atopic wheeze	Cases and controls were matched for age, sex, parental atopy, indoor allergen exposure and pet ownership, analyses were adjusted for total fat intake	Vitamin E: no association

Table 2.6: *β–carotene in relation to indicators of asthma and allergy in epidemiological studies in children*

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Harik-Khan et al.[95] 2004 US	NHANES III 4093 children 6-17 yrs Cross-sectional	- Serum α-carotene (μg/dl), - Serum β-carotene (mg/dl)	Dd-asthma	Age, gender, household size, BMI, household head gender, educational, and employment status, parental asthma or hay fever, race, ETS in home, levels of serum antioxidant vitamins	- Serum α-carotene: beneficial association with asthma: OR 0.945 (0.899-0.993) - Serum β-carotene: no association with asthma
McKeever et al.[93] 2004 US	NHANES III 4428 children 6-16 yrs Cross-sectional	Serum β-carotene (mean (SD) 15.6 (9.0) g/dl)	Skin sensitisation to allergens	Age, sex, smoking, BMI, poverty index, race/ethnicity, serum levels of total cholesterol and triglycerides	Serum β-carotene: no association with skin sensitisation: OR for 1 SD difference 0.95 (0.87-1.03)
Rubin et al.[96] 2004 US	NHANES III 6153 children 4-16 yrs Cross-sectional	Serum β-carotene (mean (SD) 16.5 (10.7) g/dl)	Prevalent asthma	Age, sex, race, BMI, education level of the head of the household, household crowding, urban residence, passive and active smoking, parental history of asthma and/or hay fever, child history of hay fever, child avoidance of pets due to allergies, serum cholesterol, serum triglycerides	Serum β-carotene: no association with asthma: OR per 1 SD increase 0.87 (0.7-1.0)
Murray et al.[65] 2006 UK	37 sensitised children with recurrent wheeze, 37 controls 3-5 yrs Nested case-control	Carotene intake (mean (95% CI) cases 2794.2 (2376.8-3211.6) vs 2374.5 (2014.8-2734.2) μg/day) SFFQ	Atopic wheeze	Cases and controls were matched for age, sex, parental atopy, indoor allergen exposure and pet ownership, analyses were adjusted for total fat intake	Carotene: no association

2.6.6 Vitamin A

Vitamin A (retinol) is a lipid-soluble vitamin derived primarily from animal based foods (meat, liver, eggs, and dairy products). Retinol plays a major role in lung development and normal respiratory epithelial development as it is assumed to repair damage from inflammation to the lung epithelium cells. In the body vitamin A can be produced from β-carotene. Although β-carotene, a pro-vitamin A, has antioxidant properties, preformed vitamin A has no antioxidant properties.[111] Vitamin A builds up in the liver and can therefore be harmful at too high levels of consumption. By consumption of foods rich in carotenoids, the supply of vitamin A can be build up safely as the body will only convert as much vitamin A from carotenoids as needed.

Vitamin A intake has been beneficially associated with asthma[64] and lung function[84] in children. However, the association reported by Huang and Pan[64] was only found in univariate analysis, whereas the association reported by Gilliland and colleagues[84] was only significant in girls. Hijazi and colleagues[7] and Murray and colleagues[65] found no associations between vitamin A and asthma or (atopic) wheeze. Serum vitamin A level was harmfully associated with skin sensitization in the study of Mckeever and colleagues[93] but was not associated with asthma in the study of Harik-Kahn and colleagues[95] (table 2.7). There is no indication for a beneficial effect of vitamin A (intake or serum levels) on end-points of asthma or allergy.

2.6.7 Vitamin D

Following observations of associations between maternal vitamin D intake during pregnancy and childhood wheeze at 3[112] and 5 years of age[113] (table 2.10) it has been proposed that vitamin D deficiency in pregnancy has contributed to the increase in asthma in the Western world.[114] However, on the other side, a study of Gale and colleagues reported an adverse association of maternal serum vitamin D status during pregnancy and asthma and atopy in childhood.[115] Hypponen and colleagues found that vitamin D supplementation in infancy was associated with an increased risk of atopy and allergic rhinitis in adulthood.[116] Since, vitamin D is produced in the skin after exposure to sunlight, vitamin D status cannot be assessed by calculating the intake by food consumption or supplementation only. The results from project Viva[112] and the SEATON birth cohort[113] are promising but more convincing data is necessary before advocating large intervention studies on vitamin D supplementation during pregnancy for prevention of asthma and allergy in children, because vitamin D supplementation may have adverse effects as well.[117]

Table 2.7: *Vitamin A in relation to indicators of asthma and allergy in epidemiological studies in children*

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Hijazi et al.[7] 2000 Saudi-Arabia	114 cases and 202 controls from a population of 1444 children 12 yrs Case-control	Vitamin A intake (mean (SD) 3483 (2309) RE/day) FFQ	Asthma or wheeze in the last 12 months	-	Vitamin A: no association with asthma or wheeze (p=0.23) (uv)
Huang and Pan.[64] 2001 Taiwan	NAHSIT 1166 adolescents 13-17 yrs Cross-sectional	Vitamin A intake (quartiles) 24-h recall	Dd-asthma	-	Vitamin A: beneficial association with asthma (higher intake lower prevalence) (p-trend=0.037) (uv)
Ellwood et al.[129] 2001	ISAAC children from 53 ISAAC countries 13-14 yrs Ecological	Vitamin A intake from vegetables (ranged from 30-739 μg/day) National food intake data, Food balance sheet and food supply data	Current wheeze, sleep disturbing wheeze, allergic rhinoconjunctivitis, atopic eczema	GNP	Vitamin A from vegetables: beneficial association with current wheeze and eczema: 100 μg/day increase 1% (p=0.04) and 1% (p=0.001) decrease in current wheeze and eczema resp.
Harik-Khan et al.[95] 2004 US	NHANES III 4093 children 6-17 yrs Cross-sectional	Serum vitamin A (mg/dl)	Dd-asthma	Age, gender, household size, BMI, household head gender, education, and employment status, parental asthma or hay fever, race, ETS in home, levels of serum antioxidant vitamins	Serum vitamin A: no association with asthma
Gilliland et al.[84] 2003 US	Children's Health Study 2566 children 11-19 yrs Cross-sectional	Vitamin A intake from diet (IU/day) or from diet + supplements (lowest decile vs higher intake) FFQ	Pulmonary function: FVC, FEV_1, FEF_{25-75}, FEV_1/FVC ratio, PEF	Community, grade, spirometer, technician, pressure, log(height), age, race, asthma, respiratory illness at lf test, in utero exposure to maternal smoke, current ETS exposure, total energy intake	Vitamin A: beneficial association with FEF_{25-75} in girls; no strong association with lf in boys

Table 2.7 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
McKeever et al.[93] 2004 US	NHANES III 4428 children 6-16 yrs Cross-sectional	Serum vitamin A (mean (SD) 39.1 (9.1) μg/dl)	Skin sensitisation to allergens	Age, sex, smoking, BMI, poverty index, race/ethnicity, serum levels of total cholesterol and triglycerides	Serum vitamin A: harmful association with sensitisation: OR for 1 SD difference 1.11 (1.04-1.78)
Murray et al.[65] 2006 UK	37 sensitised children with recurrent wheeze, 37 controls 3-5 yrs Nested case-control	Vitamin A intake (mean (IQR) cases 264.0 (160.5) vs 264.0 (128.0) μg/day) SFFQ	Atopic wheeze	Cases and controls were matched for age, sex, parental atopy, indoor allergen exposure and pet ownership, analyses were adjusted for total fat intake	Vitamin A: no association

2.6.8 Selenium

Selenium is a cofactor for antioxidant enzymes like glutathione peroxidase, which reduces hydrogen peroxide and other organic peroxides and thus plays a key role in protecting cells against oxidative damage. Selenium has also an overlapping function with vitamin E. The main sources of selenium are grain, meat, seafood and certain vegetables.

No significant cross-sectional associations have been found between serum selenium levels or selenium intake and asthma, wheeze, or skin sensitization in children,[7,93,96] although there is evidence that plasma selenium levels[118] and glutathione peroxidise activity[119,120] were lower in asthmatic children compared with controls. Two longitudinal studies suggest that early selenium exposure may be important. In a birth cohort study umbilical cord selenium concentration was negatively associated with persistent wheeze in the children up to 3.5 years,[121] although the authors suggest that due to multiple analyses, the main findings might have occurred by chance.[121] Another study found that low levels of blood selenium at 6 months to 5 year of age predicted an increased risk of childhood wheeze 8 years later.[122] In the SEATON birth cohort study, maternal plasma selenium during pregnancy and neonatal cord plasma selenium were beneficially associated with childhood wheeze at 2 years of age, but disappeared at 5 years of age[123] (table 2.8).

A Cochrane review concludes that there is some indication that selenium supplementation could be a useful complement to medication for patients with asthma.[124] However, only one randomized controlled trial assessing the effect of selenium was included in this review, as no other studies met the inclusion criteria. That trial, of adults with intrinsic asthma was very small and whilst the authors reported clinical improvement in the supplemented group compared with the placebo group, based on a composite clinical improvement in individual objective measure such as lung function, BHR or peak flow.[125]

Overall, there is not enough evidence to conclude that selenium has a beneficial effect on the development of allergic disease. Evidence to date is that selenium supplementation improves asthma severity is weak, but a large UK trial has just been completed.

2.6.9 Zinc and copper

Evidence from studies investigating effects of zinc and copper intake or status on asthma or allergy in children is very limited (table 2.9). Two case-control studies in asthmatic children[126] and children with atopic wheeze[65] did not find associations with zinc or copper intake or status (serum or hair), whereas two other case control studies found beneficial associations with hair zinc levels and asthma[127] or wheeze,[128] but an adverse association with serum and hair copper level and asthma and eczema.[127]

Next to the limited evidence from these case-control studies, there is also evidence from studies on maternal zinc intake during pregnancy and childhood asthma/allergy symptoms. The study of Litonjua and colleagues in project Viva has found a beneficial association between maternal zinc intake during pregnancy and wheeze in two year old

children[110], and it was beneficially associated with ever having asthma in 5-year-old children in the SEATON study.[98]

Table 2.8: *Selenium in relation to indicators of asthma and allergy in epidemiological studies in children*

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Shaw et al.[122] 1994	708 children 8-13 yrs Nested case-control	Serum Selenium	Current wheeze	-	Serum Selenium: beneficial association with current wheeze OR low vs high level 3.1 (0.9-11.8)
Hijazi et al.[7] 2000 Saudi-Arabia	114 cases and 202 controls from a population of 1444 children 12 yrs Case-control	Selenium intake (mean (SD) cases 0.24 (0.05) vs controls 0.25 (0.06) mg/day) FFQ	Asthma or wheeze in the last 12 months	-	Selenium: no association with asthma or wheeze (p=0.56) (uv)
Kocyigit et al.[118] 2004	Asthma patients and healthy controls Case-control	Plasma selenium level	Asthma	-	Plasma selenium: beneficial association with asthma (higher in healthy subjects) (p<0.01)
McKeever et al.[93] 2004 US	NHANES III 4428 children 6-16 yrs Cross-sectional	Serum selenium (mean (SD) 117.2 (14.2) ng/ml)	Skin sensitisation to allergens	Age, sex, smoking, BMI, poverty index, race/ethnicity	Serum selenium: no association with sensitisation: OR for 1 SD difference 0.99 (0.94-1.05)
Rubin et al.[96] 2004 US	NHANES III 6153 children 4-16 yrs Cross-sectional	Serum selenium (mean (SD) 117.1 (14.3) ng/dl)	Prevalent asthma	Age, sex, race, BMI, education level of the head of the household, household crowding, urban residence, passive and active smoking, parental history of asthma and/or hay fever, child history of hay fever, child avoidance of pets due to allergies	Serum selenium: no association with asthma: OR per 1 SD increase 0.88 (0.7-1.1)

Table 2.8 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Murray et al.[65] 2006 UK	37 sensitised children with recurrent wheeze, 37 controls 3-5 yrs Nested case-control	Selenium intake (GM (95% CI) cases 46.7 (41.6-52.4) vs 45.4 (40.4-51.0) μg/day) SFFQ	Atopic wheeze	Cases and controls were matched for age, sex, parental atopy, indoor allergen exposure and pet ownership, analyses were adjusted for total fat intake	Selenium: no association

Table 2.9: *Copper and zinc in relation to indicators of asthma and allergy in epidemiological studies in children*

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Goldey et al.[126] 1984	29 asthma patients, 11 prednison-treated asthma patients, 21 non-asthmatics 6-20 yrs Case-control	- Serum zinc and copper level - Hair zinc and copper level - Zinc and copper intake from diet	Asthma	-	Zinc and copper: no association with asthma
Di Toro et al.[127] 1987	43 atopic children (22 asthmatics, 21 eczematous), 19 healthy children 2-14 yrs Case-control	- Serum zinc and copper level - Hair zinc and copper level - Zinc and copper intake from diet	Asthma, eczema	-	- Serum zinc: no association with asthma/eczema - Serum copper level: harmful association with asthma (higher in asthmatics) ($p<0.05$) 2. Hair zinc: beneficial association with asthma (higher in healthy children) ($p<0.05$) - Hair copper: harmful association with asthma and eczema (higher in asthmatic and eczematous children) - Zinc and copper intake: no association with asthma or eczema
Murray et al.[65] 2006 UK	37 sensitised children with recurrent wheeze, 37 controls 3-5 yrs Nested case-control	- Zinc intake (mean (95% CI) cases 7.8 (7.0-8.5) vs 7.4 (6.8-7.9) mg/day) - Copper intake (mean (95% CI) cases 0.72 (0.65-0.78) vs 0.69 (0.63-0.76) mg/day) SFFQ	Atopic wheeze	Cases and controls were matched for age, sex, parental atopy, indoor allergen exposure and pet ownership, analyses were adjusted for total fat intake	- Zinc: no association - Copper: no association
Tahan and Karakukcu[128] 2006 Turkey	34 wheezy children, 14 controls Case-control	Hair zinc level (μg/g)	Wheeze	-	Hair zinc level: beneficial association with wheeze ($p<0.001$) (uv)

Table 2.10: *Maternal diet or supplementation during pregnancy in relation to asthma and/or allergy in children*

Authors	Study characteristics	Assessment of intake or intervention during pregnancy	Asthma/allergy end-point	Confounders	Key results
Devereux et al.[109] 2002 UK	SEATON 2000 pregnant women, 223 neonatal samples Cohort	- Maternal vitamin E intake calculated from FFQ - Maternal serum vitamin E level at 16-20 weeks gestation	CBMC proliferative responses to con A, *Mycobacterium tuberculosis*, PPD, timothy grass pollen, extract, house dust mite extract	- - Serum cholesterol, serum total lipids	- Vitamin E intake: beneficial association with CBMC-proliferative responses; higher intake→reduced responses to timothy grass pollen and house dust mite - Serum vitamin E: no association
Mihrshahi et al.[44] 2003 Peat et al.[45] 2004 Marks et al.[46] 2006 Australia	CAPS 616 unborn children, 554 children followed up at 18 months, 254 at 3 years and 516 at 5 years of age Intervention study	Fish oil supplementation (184 mg n-3 PUFA /day) and provision of oils and spreads low in n-6 and high in n-3 for use in food preparation or placebo supplementation with capsules containing 83% MUFA oils, provision of oils and margarines high in n-6 fatty acids	Asthma, cough, wheeze, eczema, atopy to inhaled and food allergens	-	- n-3 PUFA supplementation: beneficial association with wheeze at 18 months of age - n-3 PUFA supplementation and n-6 restriction: beneficial association with atopic cough at 3 yrs of age; no association with asthma, wheeze, eczema or atopy at 5 yrs of age
Dunstan et al.[47,48] 2003 Australia	98 atopic pregnant women RCT	Fish oil supplementation (3.7 g n-3 PUFA/day) or placebo from 20 weeks gestation until delivery	Neonatal cytokine responses and IgE levels, atopy at 1 yr of age	-	Fish oil supplementation: lower neonatal cytokine responses; beneficial association with atopy at 1 yr: OR 0.34 (0.11-1.02)

Table 2.10 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Shaheen et al.[121] 2004 UK	ALSPAC 14,541 pregnant women; 13,971 children followed up at 1 yr of age Cohort	Umbilical cord blood concentrations of selenium, zinc, copper, manganese, magnesium, iron, lead and mercury	Wheeze at 30-42 months, transient, late-onset and persistent wheeze from 0-42 months, and eczema at 18-30 months	Maternal: age at birth, parity, smoking during pregnancy, educational level, housing tenure, ethnic origin, Child's: sex, birth weight	- Cord selenium: beneficial association with persistent wheeze OR per doubling conc. 0.67 (0.45-0.99) - Cord iron: beneficial association with late-onset wheeze and eczema: OR 0.86 (0.75-0.99) and 0.90 (0.83-0.98) resp.
Martindale et al.[97] 2005 UK	SEATON 2000 pregnant women; Follow-up in children at 2 years of age Cohort	- Vitamin E, C, β-carotene, selenium, magnesium, manganese, copper, and zinc intake calculated from FFQ during pregnancy (quintiles) - Plasma a-tocopherol, ascorbate and β-carotene concentration during pregnancy	Wheeze and eczema	Sex, maternal age, paternal social class, maternal atopy, maternal smoking, other children in the home and antibiotic use	- Vitamin E intake: beneficial association with wheeze without cold: OR highest vs lowest tertile 0.49 (0.26-0.93), and eczema in children from atopic mothers: OR highest vs lowest quintile 0.42 (0.22-0.82) - Vitamin C intake: harmful association with ever wheeze and eczema: OR highest vs lowest quintile 3.00 (1.47-6.12) and 1.56 (0.99-2.45) resp. - β-carotene, Mn, Mg, Cu and Zn intake: no associations
Salam et al.[49] 2005 USA	Children's Health Study 279 cases and 412 control children at 5 years of age Nested case-control	Fish intake (oily fish, non-oily fish, fish sticks, canned fish) during pregnancy, retrospectively assessed by interview	Asthma	Maternal asthma, race/ethnicity, maternal age and education, gestational age, nr of siblings, breastfeeding, mutually adjusted for the other fish variables	- Oily fish consumption: beneficial association with early persistent asthma OR $\geq$1 time/month vs never 0.45 (0.23-0.91) - Fish stick consumption: harmful association with any asthma: OR 2.04 (1.18-3.51)

Table 2.10 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Litonjua et al.[110] 2006 USA	Project Viva 1290 mother-child pairs at 2 years of age	Maternal intake of vitamin C, E, zinc, lutein+zeaxanthin, folic acid, α-carotene, β-carotene, β-cryptoxanthin, lycopene, copper during pregnancy calculated from FFQs (quartiles)	Wheeze, recurrent wheeze and eczema	Sex, maternal age, maternal and paternal asthma, family income, passive smoke exposure, breastfeeding, other children <12 yrs in the home, body weight, maternal prepregnancy BMI	- Vitamin E intake: beneficial association with any wheeze and recurrent wheeze in the first 2 yrs: OR highest vs lowest quartile 0.70 (0.48-1.03) and 0.49 (0.27-0.90) resp. - Zinc intake: beneficial association with any wheeze: OR 0.59 (0.41-0.88) - Vitamin C, lutein+zeaxanthin, folic acid, α-carotene, β-carotene, β-cryptoxanthin, lycopene, copper intake: no consistent associations
Calvani et al.[51] 2006 Italy	295 children of allergic mothers, 693 children of non-allergic mothers, 5 yrs of age Retrospective cohort	Maternal intake of fish, butter and margarine during pregnancy, retrospectively assessed	Atopy (skin prick test)	Age, occupation, eczema	- Fish intake: beneficial association with skin sensitisation to food allergens OR 2-3 times/week vs $\leq$1 time/month 0.23 (0.08-0.69) in children from non-allergic mothers - Butter intake: no associations with atopy - Margarine intake: no associations with atopy

Table 2.10 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Devereux et al.[98] 2006 UK	SEATON 2000 pregnant women, Follow-up in children at 5 years of age Cohort	- Vitamin E, C, β-carotene, magnesium, copper, zinc and iron intake calculated from FFQ during pregnancy (quintiles) - Plasma α-tocopherol concentration during pregnancy	Wheeze and asthma symptoms, spirometry, bronchodilator response, atopic sensitisation, FeNO at 5 years of age	Maternal: atopy, age, smoking, termination of full-time education, breast feeding, season of last menstrual period, mutually intake of vit E, C and/or zinc during pregnancy Paternal: social class, deprivation index Child's: sex, antibiotic use in the 1st yr, birth weight, birth order, birth crown-heel length and head circumference	- Vitamin E intake: beneficial association with current wheeze OR per intake quintile 0.82 (0.71-0.95), ever asthma 0.84 (0.72-0.98), current asthma and wheeze 0.79 (0.65-0.95), persistent wheeze 0.77 (0.63-0.93) - Plasma α-tocopherol: beneficial association with post-bronchodilator FEV1 (p=0.04) - Zinc intake: beneficial association with ever asthma OR 0.83 (0.71-0.78) and active asthma 0.72 (0.59-0.89) - Vitamin C, β-carotene, Mg, Cu and Fe intake: no associations
Camargo Jr et al.[112] 2007 USA	Project Viva 1194 mother-child pairs at 3 years of age	Vitamin D intake calculated from FFQ during pregnancy (quartiles)	Recurrent wheeze, respiratory infection, eczema	Sex, birth weight, income, maternal age, prepregnancy BMI, passive smoking exposure, breast feeding duration, nr of children <12 yrs in household, maternal and paternal history of asthma	Vitamin D intake: beneficial association with recurrent wheeze: OR highest vs lowest quartile 0.38 (0.22-0.65) sig. p-trend

Table 2.10 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Sausenthaler et al.[52] 2007 Germany	LISA 3097 children enrolled at birth, 2642 children followed up at 2 yrs of age Cohort	FFQ about consumption during last 4 weeks of pregnancy, administered shortly after birth	Doctor-diagnosed eczema, atopy (total and specific IgE) at 2 years of age	Study area, sex, maternal age at delivery, smoking during pregnancy, parental education, breastfeeding, family history of atopy, season of birth, and all dietary variables	- Margarine and vegetable oil intake: harmful association with eczema - Fish intake: beneficial association with eczema - Celery and citrus fruit intake: harmful association with sensitisation to food allergens - Deep-frying vegetable fat, raw sweet pepper, and citrus fruit intake: harmful association with sensitisation to inhalant allergens
Devereux et al.[113] 2007 UK	SEATON 2000 pregnant women, Follow-up in children 5 years of age Cohort	Vitamin D intake calculated from FFQ during pregnancy (quintiles)	Wheezing symptoms, spirometry, bronchodilator response, atopic sensitisation, FeNO at 5 years of age	Maternal: atopy, age, smoking, termination of full-time education, breast feeding, season of last menstrual period, intake of vit E, zinc and calcium during pregnancy Paternal: social class, deprivation index Child's: sex, antibiotic use in the 1st yr, birth weight, birth order	Vitamin D intake: beneficial association with ever wheeze, current wheeze and persistent wheeze: OR highest vs lowest quintile 0.48 (0.25-0.91), 0.35 (0.15-0.85) and 0.33 (0.11-0.98) resp. and bronchodilator response (p=0.04); no associations with spirometry or FeNO
Romieu et al.[53] 2007 Menorca, Spain	507 pregnant women, 462 children followed up for 6.5 years Cohort	Fish intake score (times per week log transformed) from FFQ during pregnancy	Eczema at 1 yr, Atopy at 4 (IgE house dust mite) and 6 yrs (skin prick test), persistent and atopic wheeze at 6 yrs of age	Type of fish, smoking during pregnancy, birth weight, gender, gestational age, maternal atopy and social class	Fish intake: beneficial association with eczema at 1 yr: OR per unit increase 0.73 (0.55-0.98), atopy at 6 yrs (positive spt for HDM): OR 0.68 (0.46-1.01) and atopic wheeze at 6 yrs: OR 0.55 (0.31-0.96)

Table 2.10 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Devereux et al.[123] 2007 UK	SEATON 2000 pregnant women, Follow-up in children at 1, 2 and 5 years of age Cohort	Plasma selenium and erythrocyte glutathione peroxidase (GPx) conc. in maternal blood during pregnancy and in neonatal cord blood	Wheeze and asthma	Maternal: atopy, smoking during pregnancy, age at recruitment, parity, plasma ascorbate Child's: gender, birth weight, birth crown-heel length and head circumference, antibiotic use in the 1st year and the Scottish Deprivation index.	- Maternal plasma Se: beneficial association with wheeze at 2 but not at 1 or 5 yrs of age - Cord plasma Se: beneficial association with wheeze at 2 but not at 1 or 5 yrs of age - Maternal and cord GPx conc.: no associations with wheez or asthma outcomes at 2 or 5 yrs of age
Fitzsimon et al.[50] 2007 Ireland	1001 pregnant women, 631 children followed up at 3 yrs of age Cohort	FFQ during pregnancy (quartiles)	Asthma from GP records	Birth weight, sex, smoke exposure, added fat, GMS status	- Oily fish consumption: beneficial association with asthma - Added or spreadable fat intake: harmful association with asthma - Fruit and vegetable consumption: beneficial association with asthma: OR 4th quartile vs 1st quartile 0.42 (0.18-0.99)

Table 2.10 Continued

Authors	Study characteristics	Assessment of intake	Asthma/allergy end-point	Confounders	Key results
Chatzi et al.[34] 2008 Menorca, Spain	507 pregnant women, 468 children followed up at 6.5 yrs of age Cohort	42-item FFQ during pregnancy and Mediterranean Diet Score	Persistent wheeze, atopic wheeze and atopy (skin prick tests) at 6.5 yrs	Maternal: age at pregnancy, asthma, atopy, social class and education, smoking during pregnancy, supplement use during pregnancy, breastfeeding Paternal asthma Child's gender, lower track resp. infections, birth weight, gestational age, birth order, number of siblings and BMI at 6.5 yrs	- Fish intake: beneficial association with persistent wheeze: OR >2-3 times/week vs ≤2-3 times/week 0.34 (0.13-0.84) - Vegetable intake: beneficial association with persistent wheeze and atopy: OR >8times/week vs ≤8 times/week 0.36 (0.14-0.92) and 0.40 (0.22-0.72) resp. - Legume intake: beneficial association with persistent wheeze: OR >1 time/week vs ≤1 time/week 0.36 (0.13-1.01) - High Mediterranean diet score: beneficial association with persistent wheeze, atopic wheeze and atopy
Gale et al.[115] 2008 UK	596 pregnant women, 440 children followed up at 9 months and 178 at 9 years Cohort	Serum vitamin D concentration during pregnancy	Eczema at 9 months, asthma at 9 years of age	-	Serum vitamin D: harmful association with eczema at 9 months of age and asthma at 9 years of age: OR >75 nmol/l vs <30 nmol/l 3.26 (1.15-9.29) and 5.40 (1.09-26.65) resp.

2.7 Gaps and opportunities

Diet has a profound effect on health, but the extent to which it influences allergy and asthma is still unclear. The literature is fragmentary and hard to summarize in a systematic way and difficulties with many small studies leave unexplained contradictions in the literature. Nutritional epidemiology is particularly subject to error due to confounding. Not only is diet likely to be associated with other potential risk factors, but also nutrients in the diet themselves are likely to be correlated with each other, making it difficult to distinguish which nutrient is important in a particular instance. Despite these difficulties a number of dietary hypotheses have been developed to explain some of the variation in asthma, and to a lesser extent, allergy. The main dietary hypotheses until now relate to dietary antioxidants, lipids and electrolytes. Further elucidation of these hypotheses will be important for their public health and clinical implications, in particular the way in which doctors treat and advise patients with allergic diseases, and for our understanding of the underlying mechanisms of these diseases.

Wide variations in the prevalence of asthma and in the quality of diet across Europe make this an excellent environment in which to support multi-centre studies on diet and its effect on allergy and asthma. The use of multicentre designs also provides the ability to check the consistency of findings in different environments. The early onset of asthma and its persistence has led to considerable interest in the possibility of early programming, that is early and irreversible change due to exposure in a vulnerable period, and one of these exposures could be diet of the mother or the child during early life. Recently, the number of observational studies on the potential role diet in fetal and infant life has grown considerably, with promising results. Observational studies, though essential are unlikely, however to provide the final evidence on the effects of diet on asthma and allergy. Additionally, genetic epidemiology could provide an opportunity to investigate possible interactions between genes and nutrient intake, whereas intervention as well as animal studies would be useful to clarify underlying mechanisms.

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Chapter 3

Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms

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Abstract

Background We have previously reported associations between maternal vitamin E, vitamin D and zinc intakes during pregnancy and asthma, wheeze and eczema in 5-year-old children. In this report we investigate whether maternal intake of specific foods during pregnancy is associated with asthma and allergic outcomes in the same children.

Methods A longitudinal birth cohort study was conducted among 1924 children born to women recruited during pregnancy. Maternal diet during pregnancy was assessed by food frequency questionnaire (FFQ). Cohort children were followed up at 5 years by symptom questionnaire and FFQ. Food groups of interest were fruit, vegetables, fruit juice, whole grain products, fish, dairy products and fat spreads. Trends across outcome groups defined by level of food intake are presented.

Results 1253 children participated at 5 years, maternal FFQ data were available for 1212. No consistent associations were found between childhood outcomes and maternal intake of the analysed foods, except for apples and fish. Maternal apple intake was beneficially associated with ever wheeze (OR highest vs lowest tertile 0.63; 95% CI 0.42-0.95), ever asthma (OR 0.54; 0.32-0.92) and doctor-confirmed asthma (OR 0.47; 0.27-0.82) in the children. Maternal fish consumption was beneficially associated with doctor-confirmed eczema (OR $\geq$1/week vs never 0.57; 0.35-0.92).

Conclusion There was no evidence for associations between maternal intake of most foods during pregnancy, and asthma, respiratory and allergic outcomes in 5-year-old children, except for apples and fish. Consumption of apples and fish during pregnancy may have a protective effect against the development of childhood asthma and allergic disease.

3.1 Introduction

Can mothers protect their children from developing asthma or atopic disease through what they eat during pregnancy? The answer to this question is important because of the potential for intervention to prevent childhood asthma and atopic disease. There is some evidence that lifestyle and dietary habits can influence the development of childhood asthma and allergy *in utero*.[1–3] We have established a birth cohort to investigate whether maternal diet during pregnancy is associated with childhood asthma and atopic disease. We have reported associations between maternal vitamin E intake during pregnancy and cord blood mononuclear cell (CBMC) responses at birth, wheezing in the second year of life and wheeze, asthma, ventilatory function, and exhaled nitric oxide at age 5 years.[2–4] We have also reported associations between maternal intakes of vitamin D and zinc and wheeze, asthma, and eczema outcomes in the same children.[5]

To date, nearly all reports relating maternal diet during pregnancy to childhood asthma and atopy have focused on individual nutrients,[2,4–7] little data exists relating maternal intake of specific foods during pregnancy and the subsequent development of childhood asthma and atopic disease. Our birth cohort provides an opportunity to examine associations between childhood respiratory/atopic outcomes and maternal intake of individual foods during pregnancy, particularly those foods (fruit, vegetables, whole grain products, and fish) that are rich sources of the nutrients (vitamin E, vitamin D, and zinc) that we have previously reported to be associated with childhood outcomes.[2–5] A second group of foods of particular interest are those that have previously been reported to be associated with asthma or atopic disease in adults and children (citrus fruit, apples, fruit juices, vegetables, fish, oily fish, dairy products, and whole grain products). Beneficial associations in children have been reported for fruit,[8–10] fruit juice,[11] vegetables,[8] fish,[9,12] oily fish,[12] full fat dairy products,[13] and whole grain products,[13,14] while harmful associations have been reported for margarine and salt.[15,16]

Advantages of looking at foods are that they contain a mixture of micronutrients that may contribute more than the sum of their parts, and that it examines associations with micronutrients that may be currently unrecognised or not easily quantifiable. In addition, an evaluation of the associations with nutrients and foods will guide any future intervention study that could be the basis for public health intervention to prevent asthma and atopic disease by dietary intervention.

3.2 Methods

3.2.1 Study population and study design

Details of the study design have been published previously.[2–4] Briefly, 2000 healthy pregnant women were recruited between October 1997 and April 1999 irrespective of their asthma/atopic status, while attending antenatal clinics at Aberdeen Maternity Hospital at a median gestational age of 12 weeks. Apart from expected slight biases the recruited women were representative of the local obstetric population.[4] At en-

rolment, the women completed an interviewer-administered questionnaire and atopic status was ascertained by skin prick testing (ALK-UK, Hungerford, UK). Although the ensuing cohort of singleton children was followed up at 1, 2 and 5 years, this report is limited to the 5–year follow–up.

3.2.2 Child's health outcomes

At 5-years a questionnaire based on the ISAAC core questions on symptoms of asthma, allergic rhinitis and atopic eczema was sent to all participating families, with up to two reminders.[2,4,17] The main questionnaire-derived outcomes of interest were "Has your child ever wheezed?" (with supplementary questions about wheeze in the last year), "Has your child ever suffered from asthma?", "Has this been confirmed by a doctor?", and "Has your child received treatment for asthma in the past 12 months?". Similar questions enquired about eczema and hay fever. Current asthma was defined as asthma and wheeze in the previous year.

Responding parents were also invited to complete a food frequency questionnaire (FFQ) pertaining to the study child's current diet and to bring the child to the hospital for an assessment including spirometry, skin prick testing, and measurement of exhaled nitric oxide (FENO). Originally, measurement of bronchodilator response and FENO were not included in the study protocol but introduced for the last 510 and 262 children respectively.[2] Details of the hospital assessment have been published previously.[2] Spirometry was measured using a pneumotachograph (Spirotrac IV version 4.22, Vitalograph, Maids Moreton, UK). Bronchodilator response was expressed as percentage change in FEV_1, 15 minutes after inhalation of 400 g salbutamol. Atopy was defined as at least one positive skin prick test response to the allergens: cat, timothy grass, egg, and house dust mite (ALK-UK, Hungerford, UK). A positive response was defined as a mean wheal diameter of 3 mm or greater than the negative control. A NIOX analyser (Aerocrine-AB, Solna, Sweden) was used to measure FENO. Parents gave their written consent and the Grampian Research Ethics Committee approved the study.

3.2.3 Assessment of maternal diet during pregnancy

Maternal diet during pregnancy was assessed by a semi-quantitative FFQ (version 5.4 of the Scottish Collaborative Group FFQ) mailed at 32 weeks gestation.[18] This FFQ consisted of 150 items, divided into 20 food groups. Mothers were asked to describe how much of each food on the list they had eaten in the previous 2-3 months. Categories for the frequency of the foods eaten were 'R' for rarely or never, 'M' for once or twice a month, and the categories 1 to 7 days per week. Further to this categorisation the amount of each food consumed ranged from 1 to 5+ measures per day. For each food a measure was specified, which was usually a small unit (e.g. slice), or household measure (e.g. tablespoon), rather than a typical portion. In order to convert to a numerical scale the frequency value 'R' was replaced by 0 and the value 'M' by 0.375. The number of measures per day was multiplied by the number of days per week to obtain the total measures per week. The food groups

of interest in this study were total fruit, citrus/kiwi fruit, apples, total vegetables, green leafy vegetables, pure fruit juice, whole grain products, total fish, total oily fish, total fat from dairy products, and exclusive butter versus margarine/low fat spread used as spread. The individual foods included in the food groups are described in table 3.1.

Table 3.1: *Constituent foods of the food groups used for analyses*

Food group	Constituent foods
Total fresh fruit	Apples, bananas, oranges, pears, peaches and nectarines, kiwi fruit, all other fruit (grapes, strawberries, melon, plums, etc.)
Citrus/kiwi fruit	Oranges, kiwi fruit
Total vegetables	Peas and green beans, carrots, cabbage and swede, broccoli, spinach and spring greens, leeks and courgettes, sweetcorn, onions, tomatoes, sweet peppers, other cooked vegetables (cauliflower, sprouts, etc.) and other salad vegetables (lettuce, cucumber, etc.)
Green leafy vegetables	Broccoli, spinach and spring greens, leeks and courgettes, and other cooked vegetables (cauliflower, sprouts, etc.)
Pure fruit juice	Pure fruit juices (orange, apple, etc.)
Whole grain products	Wholemeal bread, bran flakes/sultana bran/all bran, shredded wheat/weetabix, muesli, porridge, brown rice
Total fish	Fish fingers, fried white fish (cod, haddock, plaice, etc.), grilled steamed or baked white fish, fried oily fish (salmon, herrings, mackerel), grilled steamed or baked oily fish, smoked fish (haddock, mackerel, kippers, etc.), tinned sardines, tinned tuna
Oily fish	Fried oily fish (salmon, herrings, mackerel), grilled steamed or baked oily fish, smoked fish (haddock, mackerel, kippers, etc.)
Dairy products	Milk, low fat yoghurt, full fat yoghurt, low calorie yoghurt, fromage frais, cream, full fat cheddar-type cheese, medium fat cheese, full fat cream cheese, low fat cream cheese, cottage cheese, butter

The total number of measures per week was divided into tertiles for total fruit, citrus/kiwi fruit, apples, total vegetables, green leafy vegetables, pure fruit juice, whole grain products and into the categories never, less than once a week, and once or more a week for total fish and total oily fish. To facilitate extrapolation to the general population, subdivisions of food intakes into tertiles were derived from all of the women completing the FFQ and not merely those responding at 5 years.

3.2.4 Assessment of child's diet at age 5 years

Parents were invited to complete an FFQ to assess the study childs dietary intake over the previous 3 months. Version C1 of the Scottish Collaborative Group FFQ was used. This is a 121 item semi-quantitative instrument based on the questionnaire used for the mothers in pregnancy, but has been modified for use in pre-school children aged 3–5 years by simplifying the response choices and changing the food list and portion sizes to be appropriate for pre–school children. Validity for specific nutrients in this age group has been reported.[19]

3.2.5 Statistical analyses

All analyses were carried out using SPSS Version 13.0 (SPSS Inc, Chicago, USA). Mantel-Haenszel odds ratios were used to assess the univariate associations between childhood outcomes and food groups, while ordinary least squared multiple logistic regression analysis using forced choice selection to adjust for covariates was used for the multivariate analyses. Covariates in the multivariate model were maternal age, paternal social class, maternal age of leaving full time education, maternal smoking during pregnancy, maternal asthma (for wheeze, asthma, and hay fever outcomes), maternal atopy, child's birth weight, child's sex, presence of older siblings, breast feeding and smoking in the child's home at 5 years.

3.3 Results

3.3.1 Study population and prevalence of respiratory and allergic symptoms

Two thousand pregnant women were recruited, of whom 1751 completed the FFQ. There were 1924 live singleton births. Symptom questionnaire data at age 5 were obtained for 1253 children and maternal FFQ data were available for 1212 of these. FFQ, spirometry, bronchodilator response, skin prick test and exhaled nitric oxide results were available for 1120, 639, 238, 700 and 167 children, respectively. Maternal intake of the food groups during pregnancy and for those responding at 5 years is shown in table 3.2.

Although the mothers responding to the 5-year questionnaire were more likely to eat fruit, citrus/kiwi fruit, green leafy vegetables, whole grain products and oily fish during pregnancy than non-responding mothers, the magnitude of the differences was small (<10%). Responding mothers also had significantly lower energy intake and a lower intake of fat from dairy products. The characteristics of the mothers and children who did or did not attend the hospital for assessment has been published previously.[2] Briefly, the mothers and children who attended for hospital assessment were broadly representative of those responding to the questionnaire, with any significant differences being of small magnitude.[2] The characteristics of the mothers and their children and the prevalence of asthma and atopic symptoms in both are shown in table 3.3.

Table 3.2: *Dietary characteristics of all mothers and of mothers responding to the 5–year questionnaire*

Maternal dietary intake	**Mothers completing FFQ* during pregnancy N=1751**	**Respondent mothers at age 5 N=1212**	**p–value†**
Energy intake (kJ/day) (geometric mean, 95% CI)	10137 (9987–10290)	9981 (9815–10149)	0.006
Total fruit intake (servings/day) (mean, 95% CI)	2.22 (2.16–2.27)	2.27 (2.20–2.34)	0.008
Apple intake (measures/week) (mean, 95% CI)	3.58 (3.41–3.76)	3.59 (3.39–3.80)	0.697
Citrus/kiwi fruit intake (measures/week) (median, 25 to 75th percentiles)	1.38 (0.38 to 4.00)	1.38 (0.38 to 4.00)	0.040‡
Total vegetable intake (servings/day) (mean, 95% CI)	1.93 (1.88–1.98)	1.95 (1.89–2.01)	0.385‡
Green leafy vegetable intake (measures/week) (median, 25 to 75th percentiles)	4.38 (1.50 to 9.00)	4.50 (1.75 to 9.00)	0.013‡
Fruit juice intake (measures/week) (median, 25 to 75th percentiles)	5.00 (1.00 to 10.00)	5.00 (1.00 to 10.00)	0.146‡
Whole grain product intake (portions/week) (median, 25 to 75th percentiles)	3.73 (0.38 to 8.18)	4.13 (0.38 to 8.67)	$\leq$0.001‡
Total fish intake (portions/week) (median, 25 to 75th percentiles)	1.58 (0.67 to 3.00)	1.67 (0.75 to 3.08)	0.054
Oily fish intake (portions/week) (median, 25 to 75th percentiles)	0.00 (0.00 to 0.50)	0.00 (0.00 to 0.50)	0.002
Fat from dairy products intake (g/week) (mean, 95% CI)	154 (149–159)	151 (145–157)	0.070
Exclusively butter vs margarine/low fat spread	20.5% vs 79.5%	21.6% vs 78.4%	0.148

*FFQ, food frequency questionnaire.

†Responders at age 5 vs non-responders.

‡Non-parametric test.

Table 3.3: *Characteristics of mothers and children and prevalence of respiratory and atopic symptoms*

Maternal characteristics	**N=1253**	**Children's characteristics at age 5**	**N=1253**
Age at recruitment (mean, 95% CI)	29.9 (29.6–30.2)	Male (n, %)	630 (50.3)
Partners social class non-manual (n, %)	767 (61.2)	Birth weight (g) (mean, 95% CI)	3458 (3426–3489)
Age left full time education (median, IQR)	18.5 (16.0 to 21.0)	Ever breast fed (n, %)	931 (74.3)
Pregnancy FFQ returned (n, %)	1212 (96.7)		
Smoking during pregnancy (n, %)	288 (23.0)		
Prevalence of respiratory and atopic symptoms in mothers	**n (%)**	**Prevalence of respiratory and atopic symptoms in children**	**n (%)**
Ever wheezed	415 (33.1)	Wheeze in last 12 months	162 (12.9)
Current asthma	125 (10.0)	Wheeze without cold in last 12 months	84 (6.7)
Ever had asthma	189 (15.1)	Ever wheezed	253 (20.2)
Asthma medication	123 (9.8)	Asthma and wheeze in last 12 months	107 (8.5)
Ever had hay fever	313 (25.0)	Doctor confirmed asthma	145 (11.6)
Ever had eczema	213 (17.0)	Ever had asthma	156 (12.5)
Atopic sensitisation	448 (35.8)	Doctor confirmed eczema	380 (30.4)
		Current eczema treatment	191 (15.3)
		Ever had eczema	406 (32.4)
		Doctor confirmed hay fever	68 (5.4)
		Current hay fever medication	44 (3.5)
		Ever had hay fever	111 (8.9)
		Atopic sensitisation (N=699)	149 (21.3)

3.3.2 Associations between maternal diet during pregnancy and respiratory and atopic symptoms in children aged 5 years

Recruitment data were available for all 1253 mother-child pairs but, because of incomplete ascertainment at the ensuing data collection points, the number of mother-child pairs with complete datasets included in the final multivariate analyses was less than the total number of respondents at age 5 (N=1253). For the 1253 mother-child pairs, FFQ data at 32 weeks gestation were available for 1212, birth weight data were available for 1200, and breast feeding and maternal age of leaving full time education data collected at 1 year were available for 1176 and 1113, respectively. Incomplete mother-child pairs appeared to be random, and those with complete datasets did not differ significantly from those with incomplete datasets for all the variables included in the multivariate analyses.

No consistent linear associations were found between maternal intake of total fruit, citrus/kiwi fruit, total vegetables, green leafy vegetables, fruit juice, whole grain products, fat from dairy products or butter versus margarine/low fat spread use and respiratory or atopic outcomes in the 5-year-old children, nor were there consistent associations between maternal intake of food groups and spirometry, atopic sensitisation, bronchodilator response or exhaled nitric oxide.

Beneficial associations were found between maternal apple intake and the childhood outcomes of ever wheezed, ever had asthma and doctor confirmed asthma at age 5, with significant linear trends (table 3.4).

Maternal apple intake was not associated with childhood eczema and hay fever or atopic sensitisation. However, beneficial associations were found between maternal total fish intake and doctor confirmed eczema and currently treated eczema (table 3.5), and between maternal oily fish intake and doctor confirmed hay fever (table 3.6). There was a significant beneficial association between higher maternal oily fish intake ($\geq$1 portion a week) and ever having hay fever, but the association did not show a significant linear trend (p=0.159, table 3.6).

Inclusion of maternal supplement use during pregnancy did not alter the magnitude or significance of the associations between maternal food intake and outcomes in 5-year-old children.

3.3.3 Association between the child's diet and respiratory and atopic symptoms at age 5 years

Maternal diet during pregnancy and the child's diet at age 5 were weakly but significantly positively correlated. Kendall's tau-b statistics were 0.21 ($p<0.001$) for the child's and maternal total fruit consumption, 0.15 ($p<0.001$) for apple consumption, and 0.20 ($p<0.001$) and 0.26 ($p<0.001$) for total fish and oily fish consumption, respectively. Despite this, we found no consistent associations between the children's intake of apples, total fish or oily fish and respiratory or atopic symptoms at age 5 years.

Table 3.4: *Associations between maternal apple consumption and childhood wheeze and asthma at age 5 years*

		Maternal apple consumption			
Childhood outcome	**N**	**T1 (0–1/week) n=398 OR (95% CI)**	**T2 (1–4/week) n=427 OR (95% CI)**	**T3 (>4/week) n=384 OR (95% CI)**	**p–trend**
Wheeze in last 12 months					
Univariate	1003	1	1.09 (0.69–1.67)	0.61 (0.37–1.01)	0.066
Multivariate*	1003	1	1.08 (0.68–1.71)	0.67 (0.40–1.13)	0.156
Wheeze without cold in last 12 months					
Univariate	1003	1	1.32 (0.72–2.43)	0.64 (0.31–1.35)	0.286
Multivariate*	1003	1	1.27 (0.67–2.43)	0.70 (0.32–1.51)	0.411
Ever wheezed					
Univariate	999	1	0.86 (0.60–1.23)	0.59 (0.40–0.88)	0.009
Multivariate*	999	1	0.85 (0.58–1.24)	0.63 (0.42–0.95)	0.029
Asthma and wheeze in last 12 months					
Univariate	998	1	1.02 (0.60–1.73)	0.55 (0.29–1.03)	0.072
Multivariate*	998	1	1.03 (0.59–1.80)	0.60 (0.31–1.16)	0.148
Doctor confirmed asthma					
Univariate	998	1	0.87 (0.56–1.36)	0.46 (0.27–0.78)	0.005
Multivariate*	998	1	0.83 (0.52–1.32)	0.47 (0.27–0.82)	0.008
Ever had asthma					
Univariate	998	1	0.90 (0.58–1.38)	0.52 (0.31–0.86)	0.005
Multivariate*	998	1	0.86 (0.54–1.36)	0.54 (0.32–0.92)	0.026

*Adjusted for maternal age of leaving full time education, paternal social class, maternal age, maternal smoking during pregnancy, smoking in the home during childhood, energy intake, maternal asthma, maternal atopy, birth weight, presence of older siblings, sex of child and breastfeeding.

Table 3.5: *Associations between maternal total fish consumption and childhood eczema at age 5 years*

		Maternal total fish consumption			
Childhood outcome	**N**	**T1 (Never) n=107 OR (95% CI)**	**T2 (<1/week) n=255 OR (95% CI)**	**T3 (≥1/week) n=831 OR (95% CI)**	**p–trend**
Doctor confirmed eczema					
Univariate	979	1	0.77 (0.46–1.28)	0.60 (0.38–0.96)	0.016
Multivariate*	979	1	0.79 (0.47–1.32)	0.57 (0.35–0.92)	0.008
Current eczema treatment					
Univariate	982	1	0.85 (0.45–1.61)	0.67 (0.38–1.19)	0.111
Multivariate*	982	1	0.88 (0.46–1.67)	0.58 (0.32–1.06)	0.028
Ever had eczema					
Univariate	983	1	0.88 (0.53–1.47)	0.73 (0.47–1.16)	0.111
Multivariate*	983	1	0.91 (0.54–1.53)	0.68 (0.43–1.10)	0.050

*Adjusted for maternal age of leaving full time education, paternal social class, maternal age, maternal smoking during pregnancy, smoking in the home during childhood, energy intake, maternal atopy, birth weight, presence of older siblings, sex of child and breastfeeding.

Table 3.6: *Associations between maternal oily fish consumption and childhood hay fever at age 5 years*

		Maternal oily fish consumption			
Childhood outcome	**N**	**T1 (Never) n=629 OR (95% CI)**	**T2 (<1/week) n=414 OR (95% CI)**	**T3 (≥1/week) n=161 OR (95% CI)**	**p–trend**
Doctor confirmed hay fever					
Univariate	990	1	0.57 (0.31–1.08)	0.20 (0.05–0.85)	0.006
Multivariate*	990	1	0.66 (0.34–1.28)	0.28 (0.06–1.19)	0.043
Current hay fever medication					
Univariate	988	1	1.08 (0.53–2.22)	0.20 (0.03–1.53)	0.226
Multivariate*	988	1	1.02 (0.48–2.20)	0.19 (0.02–1.48)	0.194
Ever had hay fever					
Univariate	988	1	1.11 (0.70–1.75)	0.38 (0.15–0.98)	0.155
Multivariate*	988	1	1.11 (0.68–1.82)	0.37 (0.14–0.98)	0.159

*Adjusted for maternal age of leaving full time education, paternal social class, maternal age, maternal smoking during pregnancy, smoking in the home during childhood, energy intake, maternal asthma, maternal atopy, birth weight, presence of older siblings, sex of child and breastfeeding.

3.4 Discussion

This study investigated associations between maternal intake of different food groups during pregnancy and symptoms of asthma and atopy in children. There was no evidence of associations between asthma, respiratory or atopic outcomes in 5-year-old children and maternal intakes of total fruit, citrus/kiwi fruit, total vegetables, green leafy vegetables, fruit juice, whole grain products, fat from dairy products or butter versus margarine/low fat spread use. However, we have shown beneficial associations between maternal apple intake and childhood wheeze and asthma, and between maternal fish intake and childhood eczema and hay fever. There are some reports of beneficial effects of maternal fish consumption and maternal fish oil supplementation during pregnancy on childhood asthma and neonatal cord blood mononuclear cell responses [20,21] but, to our knowledge, our finding of the protective effects of maternal apple consumption during pregnancy on childhood wheeze and asthma is new.

In this cohort we have reported beneficial associations between maternal vitamin E intake during pregnancy and cord blood mononuclear cell responses at birth,[3] wheeze at age 2 years [4] and wheeze and asthma at age 5 years.[2] We have also found beneficial associations between maternal zinc intake during pregnancy and asthma and eczema in children at the age of 5 years, and between maternal vitamin D intake and wheeze in children at age 5 years.[2,5] One of the aims of this study was to investigate whether these associations with maternal nutrient intakes could be a consequence of associations with individual foods rich in one or several of these nutrients, with obvious implications for a potential dietary intervention during pregnancy. It would seem that the associations reported here with maternal intake of apples and fish are insufficient

to account for the associations with vitamin E, vitamin D and zinc because, in the UK, apples and fish provide less than 10% of dietary vitamin E and zinc intakes in women of this age group. In addition, the pattern of associations between vitamin E, vitamin D, zinc and childhood respiratory and allergic outcomes differed from those in the present study. In the UK there is no single major dietary source of vitamin E in women aged 25 to 49 years, with intake being evenly distributed between fat spreads (15%), cereals/cereal products (10%), potatoes/potato snacks (12–14%), vegetables (16–17%) and meat/meat products (10%).[22] In the present study the absence of any association between the usual dietary sources of vitamin E and respiratory outcomes suggests that the associations with vitamin E in a previous report [2] were unlikely to represent associations with other nutrients commonly found in foods containing vitamin E.

The present study suggests beneficial associations between maternal apple intake during pregnancy and wheeze and asthma at age 5 years. The evidence from other observational studies on childrens diet and respiratory and atopic symptoms is relatively consistent, showing beneficial effects of fruit and vegetable intake on indicators of asthma.[8–11] However, it is not clear whether these effects can be attributed to specific nutrients or that a high intake of fruit and vegetables is an indicator of a healthier lifestyle. The specific association found with apples in this study and not with total fruit, citrus, fruit juice or vegetable consumption suggests an effect specific to apples, possibly because of their phytochemical content such as flavonoids. Flavonoids are polyphenolic compounds with powerful antioxidant capacities and are associated with reduced risks of several diseases including asthma and chronic obstructive pulmonary disease.[23–25] Intake of apples as a significant source of flavonoids and other polyphenols has been beneficially associated with asthma, bronchial hypersensitivity and lung function in adults.[24,26–28] These effects are usually ascribed to the strong antioxidant capacities of apples, although there is also evidence that some polyphenolic compounds can influence cytokine gene expression by Th-cells, promoting the secretion of the Th1 cytokine interferon-γ and inhibiting secretion of the Th2 cytokine interleukin-4.[29] However, there is a lack of epidemiological evidence on the relation between the intake of flavonoids or specific flavonoidrich foods and asthma or allergy in children. Although the consumption of total fresh fruit has increased in recent years, apple consumption in the UK fell from 207 g/person/day in 1974 to 173 g/person/day in 2004/5.[30] It has also been suggested that the mineral content of fruit and vegetables declined between 1940 and 1991.[31] This could be the consequence of changes in cultivation, the use of fertilisers and the choice of fruit species that can be more easily harvested or stored. The observation of beneficial associations between maternal total and oily fish consumption and current eczema and ever hay fever at age 5 years, respectively, is consistent with earlier observations.[20,21] Dunstan et al.[21] examined the effect of fish oil supplementation during pregnancy on early developing immune responses and clinical outcomes in infants predisposed to allergic disease. Neonates born to mothers supplemented with fish oil tended to have lower cord blood mononuclear cell cytokine responses to allergens and, at 1 year of age, significantly less severe disease if they had atopic dermatitis. Salam et al.[20] studied the association between maternal fish consumption during pregnancy and childhood asthma. They

found that maternal oily fish consumption at least monthly was significantly protective for persistent asthma in 5-year-old children. Other epidemiological evidence on the effect of fish intake or fish oil supplementation on asthma or allergic diseases provided by observational and intervention studies in children is inconsistent.[32,33] It is therefore more likely that the time window for N–3 polyunsaturated fatty acids to have an effect on immune regulation and subsequent asthma and atopic disease is indeed in fetal life, and that effects are limited once allergic immune responses are established.[21]

Originally, the study population of 2000 pregnant women was demographically very similar to the local obstetric population.[4] In this study there was some evidence of response bias due to the loss to follow-up with time. Participating mothers were of higher socioeconomic status and had slightly higher consumption of fruit, green leafy vegetables, whole grain products and fish and had fewer respiratory symptoms.[2] An analysis of the wheezing symptoms of the children whose mothers responded at 2 years but not at 5 years indicated that the children with no data at 5 years were more likely to have wheezed at 2 years (not shown). This type of response bias often plays a role in cohort studies because it is known that subjects with poorer socioeconomic status and lifestyle (lower educational level, poorer diet, smoking, etc.) are more difficult to trace, and that people who suffer poor health during the follow-up period are prone to attrition.[34] However, due to this type of bias, it is more likely that the observed associations in this study are underestimated than overestimated; for instance, improved ascertainment at 5 years would have resulted in a larger proportion of wheezy children with low maternal apple consumption, which would make the observed associations between maternal apple consumption and childhood asthma/wheezing symptoms stronger (in this case odds ratios closer to zero). A limitation of FFQ–derived estimates is that they are susceptible to dietary misreporting which leads to dietary misclassification of intake and/or portion sizes. Usually this misclassification is random and it also weakens rather than augments the associations. To avoid multiple hypothesis testing we chose a restricted number of food groups based on our previous findings in this cohort and earlier reported associations,[13,14,27] the lipid hypothesis [35] and antioxidant hypothesis.[36] It is possible that the associations reported could be a consequence of the number of analyses performed. However, we consider this is unlikely because some of the associations were highly significant and the associations were clustered with food groups that have previously been associated with similar outcomes in children and adults.

The predominance of associations between maternal food intakes and doctor-diagnosed outcomes raises the possibility of ascertainment bias, whereby mothers more conscious of health issues were both more likely to follow dietary advice to eat healthily and more likely to take their unwell children to the doctor to receive a formal diagnosis. Such ascertainment bias seems unlikely because it predicts that maternal apple consumption should be adversely associated with childhood doctor-diagnosed conditions — the opposite to what we report.

The observed associations with maternal food intake during pregnancy were independent of the childhood diet because inclusion of childrens apple and fish consumption in the models did not change the results, despite maternal and childhood diet

being weakly correlated.

Published cross–sectional surveys of children have reported associations between the dietary intake of citrus, kiwi fruit and vegetables and indicators of asthma,[8–10] but the present study failed to show any consistent associations between the food intake of children aged 5 years and respiratory and atopic symptoms. The children in the present study were younger than those participating in previous studies (6–11 years), and this may account for the disparity between this and previous studies. In the present study it would appear that, until at least the age of 5 years, maternal diet during pregnancy is more influential on respiratory health than childhood diet. Further follow-up of this birth cohort will be required to determine whether the associations with maternal diet decline in older children, and whether maternal and childhood diets interact in older children.

The associations between maternal apple consumption and asthma and symptoms could represent effects on airway and immune development, while the associations between maternal (oily) fish consumption and eczema and hay fever suggest effects on Th-cell differentiation,[37] yet no associations were found with lung function measures, exhaled nitric oxide and atopic sensitisation. This could reflect a loss of power due to the smaller number of children who underwent spirometric or skin prick tests.

The results of this cohort study indicate that there were no consistent linear associations between maternal intake of total fruit, citrus/kiwi fruit, total vegetables, green leafy vegetables, fruit juice, whole grain products, fat from dairy products or butter versus margarine/low fat spread use during pregnancy and asthma, respiratory and atopic outcomes in 5-year-old children. We did, however, find some evidence for protective effects of maternal apple and fish consumption. Thus, in addition to maternal intake of vitamin E, vitamin D and zinc during pregnancy,[2–5] maternal consumption of apples and fish during pregnancy may reduce the risk of children developing asthma or atopic disease. If these results are confirmed, recommendations on dietary modification during pregnancy may help to prevent childhood asthma and allergy.

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Chapter 4

Maternal food consumption during pregnancy and the longitudinal development of childhood asthma

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Abstract

Background Maternal diet during pregnancy has the potential to affect airway development, and to promote Th2-cell responses during fetal life. This might increase the risk of developing childhood asthma or allergy. We investigated the influence of maternal food consumption during pregnancy on childhood asthma outcomes from 1 to 8 years of age.

Methods A birth cohort study consisting of a baseline of 4146 participants (1327 atopic; 2819 non-atopic mothers). These pregnant women were asked about their frequency of consumption of fruit, vegetables, fish, egg, milk, milk products, nuts and nut products during the last month. Their children were followed up until the age of 8. Longitudinal analyses were conducted to assess associations between maternal diet during pregnancy and childhood asthma outcomes over these 8 years.

Results Complete data were obtained for 2832 children. There were no associations between maternal vegetable, fish, egg, milk or milk products and nut consumption and longitudinal childhood outcomes. Daily consumption of nut products increased the risk of childhood wheeze (odds ratio [OR] daily vs rare consumption, 1.42; 95% confidence interval [95% CI], 1.06 to 1.89), dyspnea (OR, 1.58; 95% CI, 1.16 to 2.15) steroid use (OR, 1.62; 95% CI 1.06 to 2.46) and 'asthma symptoms' (OR 1.47; 95% CI 1.08 to 1.99).

Conclusion Results of this study indicate an increased risk of daily versus rare consumption of nut products during pregnancy on childhood asthma outcomes. These findings need to be replicated by other studies before dietary advice can be given to pregnant women.

4.1 Introduction

The chance of developing asthma and allergy might be determined during fetal life, because there are potential mechanisms by which environmental factors like maternal diet and life-style can influence the development of the airways and immune system of the child *in utero*.[1] Adherents of the fetal programming theory or Barker hypothesis pose that adverse events like undernutrition, smoking and infections during pregnancy can result in impaired growth in the fetus and long-term physiological or metabolic changes.[2] This concept, which was established by David Barker, who found that low birth weight was associated with elevated blood pressure and increased risk of cardiovascular mortality,[3] has been postulated to play a role in the origin of asthma and allergy as well.[4,5] Several studies have found associations between low birth weight or head circumference and asthma, respiratory symptoms or atopy.[6–8] Maternal nutrient status during pregnancy may have the potential to impair fetal airway development, or to promote neonatal T helper (Th)-cell responses to allergens being biased towards Th2-cell responses, which increase the risk of developing asthma or allergic disease in childhood.[1]

Another possible mechanism is that *in utero* allergen exposure exerts an effect on the developing fetal immune system. Research has shown fetal immune responses to allergens from 22 weeks of gestation.[9] Maternal intake of allergenic foods during pregnancy may thus increase the risk of sensitisation in the fetus and subsequent allergic disease. However, the evidence of the effectiveness of maternal dietary allergen avoidance during pregnancy for prevention of childhood allergic disease is still inconclusive.[10]

Previous studies investigating the relationship between maternal diet during pregnancy and asthma or allergic disease in the offspring have found beneficial effects of higher intake of vitamin E,[11–14] vitamin D,[15,16] zinc,[13] selenium and iron,[17] and higher consumption of fish [18–23] and apples.[22] The study of Sausenthaler and colleagues[20] has found associations between maternal intake of allergenic foods during pregnancy and sensitisation against inhalant and food allergens in childhood.

The prospective studies mentioned previously have mostly assessed associations between maternal diet during pregnancy and asthma at one specific age of the child. To our knowledge, the present study is the first to use longitudinal statistical techniques to investigate this relationship over a longer time period. The aim of this study was to investigate longitudinally the influence of maternal diet during pregnancy on the prevalence of symptoms of childhood asthma from 1 to 8 years of age.

Some of the preliminary findings of this study have been previously reported in the form of an abstract.[24]

4.2 Methods

4.2.1 Study population and study design

The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study, set up in 1996, consisted of a natural history part and an intervention part.

Details of the study design have been published previously.[25] Briefly, 10,232 pregnant women completed a validated screening questionnaire at their prenatal healthcare clinic.[26] Resulting from this screening, 2949 women were defined as atopic, whereas 7283 were defined as non-atopic. 2,779 atopic women, and 5083 non-atopic women were invited to participate in the study, of whom 1327 atopic women and 2819 non-atopic women finally agreed to participate. In the intervention part of the PIAMA study only children born to atopic mothers (high-risk children) were enrolled, whereas in the natural history part children of atopic as well as children of non-atopic mothers (low-risk children) were enrolled. The oversampling of non-atopic mothers in the natural history part of the study provides that the proportion of atopic and non-atopic mothers enrolled in the total study is the same as in the screened population. (In both populations the proportion of atopic mothers is ca. 30%). Study children were born between summer 1996 and late fall 1997, questionnaires were administered when the children were 3 months of age, and yearly from 1 to 8 years of age. Children from the intervention part, the 'high-risk' natural history part, and a random sample of the 'low-risk' natural history part, drawn at the beginning of the PIAMA study, were selected for more extensive follow-up including home visits, medical examination at 4 years of age, and more extensive medical examination at 8 years of age. The Medical Ethical Committees of the participating institutes approved of the study and all participants gave written informed consent.

4.2.2 Assessment of maternal diet during pregnancy

The pregnancy questionnaire contained questions about diet. Expectant mothers were asked: "How often did you consume 'vegetables', 'fresh fruit', 'fish', 'egg', 'milk', 'milk products', 'nuts', and 'nut products such as peanut butter' during the last month?" Answer options were: (1) never, (2) one to three times a month, (3) once a week, (4) two to four times a week, (5) more than four times a week, (6) once a day, or (7) several times per day. These frequency values were combined into three categories: 'rarely' (value 1 and 2), 'regularly' (value 3, 4 and 5) and 'daily' (value 6 and 7).

A variable 'dairy' consumption was constructed by taking the average of 'milk' and 'milk product' consumption. In the Dutch general population tree nuts as well as peanuts, almonds, Brazil nuts, cashews, macadamia, pistachios etc. are commonly regarded as nuts.

If the prevalence of 'rare' or 'daily' consumption was less than 5% it was combined with the category 'regular' consumption. For vegetables, fruit and dairy, 'daily' consumption was compared with 'regular' plus 'rare' consumption. For fish, egg and nuts, 'daily' plus 'regular' consumption was compared with the reference category 'rare' consumption. For nut products, all categories contained sufficient observations, so 'daily' and 'regular' consumption were both compared with 'rare' consumption as reference category.

4.2.3 Assessment of the child's diet at 2 years of age

Questionnaires administered from 1 to 8 years of age contained questions about the child's diet. Parents were asked to indicate from the following five options how often in the previous month the child had consumed a number of different foods and drinks: not at all; a few times, but less than once a week; 1 or 2 days/week; 3 to 5 days/week; 6 or 7 days/week. The child's dietary data on 'fruit', 'vegetables', 'fish', 'egg', 'full cream milk', 'butter' and 'peanut butter' consumption at 2 years of age were used to check for potential confounding by the child's diet.

4.2.4 Child's health outcomes

The yearly follow-up questionnaires contained the ISAAC core questions on asthma, rhinitis and eczema.[27] The main outcomes of interest for longitudinal analyses were wheeze, dyspnea, prescription of inhaled corticosteroids for respiratory problems and the composite variable 'asthma symptoms' in the last 12 months. Data on wheeze was available from 1 to 8 years of age, data on dyspnea, and steroid use from 3 to 8 years of age. The composite variable 'asthma symptoms' from 3 to 8 years of age was based on the wheeze, dyspnea and steroid use outcomes. A child was defined as having 'asthma symptoms' when the parents reported one or more attacks of wheeze, and/or one or more events of dyspnea and/or prescription of inhalation steroids for respiratory problems in the last 12 months. A child who had none of these characteristics was defined as not having 'asthma symptoms'. Other outcomes that were investigated longitudinally were wheeze and dyspnea without a cold and doctor-diagnosed asthma in the last 12 months.

Additionally, the wheeze data from 1 to 8 years of age were used to categorise the children as 'never wheezers', 'early transient wheezers', 'late-onset wheezers' or 'persistent wheezers' according to Martinez and co-workers.[28] Children who did not wheeze in the first three years of life and neither from 6 to 8 years of age were classified as 'never wheezers'; children who had at least one attack of wheeze during the first three years of life but did not wheeze from 6 to 8 years of age were classified as 'early transient wheezers'; children who did not wheeze during the first three years of life but had at least one attack of wheeze from 6 to 8 years of age were classified as 'late-onset wheezers'; and children who wheezed during the first three years of life and also from 6 to 8 years of age were classified as 'persistent wheezers'.

At 8 years of age, all children who were still participating in the study were invited for a medical examination, either at home or in hospital (more extensive follow-up). During this medical examination, blood samples were drawn for the assessment of total and allergen-specific IgE. Children were considered to be sensitised against inhalant allergens if one or more allergen specific IgE levels to house dust mite (*Dermatophagoides pteronyssinus*), cat, dog, birch (*Betula verrucosa*), grass (*Dactylis glomerata*) and fungus (*Alternaria alternata*) were equal to or higher than 0.35 IU/ml. Sensitisation to food allergens was defined as a high level of allergen specific IgE to milk or egg (also $\geq$0.35 IU/ml). IgE data at 8 years of age were present for a subgroup of 1657 children.

4.2.5 Statistical analyses

All analyses were carried out using SAS for Windows version 8.2 (SAS Institute, Cary, NC, USA). Generalised Estimating Equations (GEE) were used to assess the associations between maternal diet during pregnancy and childhood symptoms of asthma during the first eight years of life simultaneously. GEE takes into account that repeated measurements in the same individual are correlated, in order to derive correct standard errors and p-values of the estimates. A working correlation structure was used to correct for this within subject correlation.[29] Potential confounders adjusted for in the final longitudinal models were: sex, maternal education, parental allergy, maternal smoking during pregnancy, smoking in the home at age 8 years, breast feeding, presence of older siblings, birth weight, maternal overweight 1 yr after pregnancy (BMI >25 kg/m^2), maternal supplement use during pregnancy, region and study arm (intervention or natural history study).

The associations between maternal intake of different food groups during pregnancy and the different wheezing phenotypes were calculated by polytomous logistic regression analyses. The final models were adjusted for the same set of confounders as used for the GEE analyses.

Multivariate logistic regression analyses were used to investigate the associations between maternal diet during pregnancy and sensitisation to inhalant and food allergens at 8 years of age.

4.3 Results

4.3.1 Study population, maternal food consumption during pregnancy and prevalence of symptoms of asthma from 1 to 8 years of age

The initial study population consisted of 4,146 pregnant women of whom 4,112 filled in the pregnancy questionnaire. Around 80% of these mothers completed this questionnaire between the 30th and 36th week of gestation. Dietary consumption frequencies for the different foods of these mothers and mothers who completed the questionnaire before the 30th week (10%) or after the 36th week (10%) were comparable.

Of the baseline population of 4,146 mothers, 183 (4.5%) were lost to follow-up before any data on the child had been collected. The study therefore started with 3,963 newborn children. Questionnaire data from 1 to 8 years of age were obtained for 3817, 3740, 3694, 3563, 3518, 3473, 3373, and 3320 children respectively. Characteristics of the study population at baseline (N=3963) and of the participants with complete data (pregnancy questionnaire, at least one of the outcome time points and all confounders) (N=2832) included in the analyses are described in table 4.1.

Participants with complete data were more likely to have a high educational level, daily fruit and dairy intake during pregnancy, and to have breast fed their child, compared to participants who did not have complete data, furthermore they were less likely to have maternal atopy or maternal asthma, a low educational level, smoked during pregnancy or in the house at baseline, to be from region south-west, and to

participate in the intervention study. Yet, the magnitude of the differences between both populations was small (<10%). The characteristics of study population at baseline compared to the population with questionnaire follow-up data (N=3320) and IgE data (N=1657) at age 8 are shown in table 4.2.

Table 4.3 shows the frequencies of 'rarely', 'regularly' and 'daily' consumption, for the different foods eaten during pregnancy. More than 50% of the pregnant woman daily consumed vegetables, fresh fruit, milk and milk products, and rarely ate fish, nuts and nut products. We also investigated possible avoidance of potentially allergenic foods. Less than 1% of the mothers reported to have never consumed milk or milk products during the last month, whereas approximately 11% never ate nuts or nut products. Almost 30% of the mothers never ate fish, whereas almost 5% never ate eggs according to the pregnancy questionnaire data.

Prevalences of childhood symptoms of asthma from 1 to 8 years of age, calculated over total number of participants followed-up at the specific time points, are shown in table 4.4. The prevalence of wheeze and dyspnea strongly decreased, whereas the prevalence of steroid use stayed quite constant over time. The composite variable 'asthma symptoms' based on these three outcomes decreased from approximately 23% at 3 years of age to approximately 13% at 8 years of age. Prevalences of wheeze without cold, dyspnea without a cold and doctor-diagnosed asthma were lower and more constant over time. From the children who provided complete wheeze data from 1 to 8 years of age (N=3043), 1866 (61.3%) were classified as never wheezers, 731 (24.0%) as early transient wheezers, 132 (4.3%) as late-onset wheezers and 314 (10.3%) as persistent wheezers.

4.3.2 Associations between maternal food consumption during pregnancy and childhood asthma outcomes from 1 to 8 years of age

Overall estimates derived with univariate and multivariate GEE analyses of the associations between maternal diet during pregnancy and childhood wheeze, dyspnea, steroid use and the composite variable 'asthma symptoms' from 1 or 3 to 8 years of age are shown in table 4.5. There were no overall associations between maternal vegetable, fish, egg, dairy and nut consumption during pregnancy and childhood wheeze, dyspnea, steroid use or 'asthma symptoms'.

Table 4.1: *Characteristics of the population at baseline and complete cases at 8 years of age*

Maternal characteristic	**Respondents at baseline N=3963**	**Complete cases at 8 years* N=2832**
Atopy†, n (%)	1237 (31.2)	788 (27.8)
Partner atopic, n (%)	1217 (30.7)	856 (30.2)
Asthma ever, n (%)	314 (7.9)	184 (6.5)
Partner ever asthmatic, n (%)	302 (7.6)	208 (7.3)
Educational level low, n (%)	894 (22.6)	577 (20.4)
Educational level high, n (%)	1331 (33.6)	1073 (37.9)
Partner's educational level low, n (%)	973 (24.6)	666 (23.5)
Partner's educational level high, n (%)	1493 (37.7)	1188 (42.0)
Smoking during pregnancy, n (%)	696 (17.6)	444 (15.7)
Supplement use during pregnancy‡, n (%)	3285 (82.9)	2372 (83.8)
Smoking in the house by mother, father or others at baseline§, n (%)	970 (24.5)	591 (20.9)
Region north, n (%)	1231 (31.1)	886 (31.3)
Region central, n (%)	1586 (40.0)	1190 (42.0)
Region south-west, n (%)	1146 (28.9)	756 (26.7)
Part of intervention study, n (%)	781 (19.7)	450 (15.9)
Age when having child, mean (SD)	30.3 (3.9)	30.6 (3.8)
Body Mass Index¶ (kg/m^2), mean (SD)	23.3 (3.6)	23.3 (3.5)
Food intake during pregnancy		
Daily vegetable intake, n (%)	2217 (55.9)	1621 (57.2)
Daily fruit intake, n (%)	3025 (76.3)	2221 (78.4)
Daily + regular fish intake, n (%)	973 (24.6)	694 (24.5)
Daily + regular egg intake, n (%)	2631 (66.4)	1894 (66.9)
Daily dairy intake, n (%)	3335 (84.2)	2453 (86.6)
Daily + regular nuts intake, n (%)	1322 (33.4)	984 (34.8)
Daily nut product intake, n (%)	243 (6.1)	169 (6.0)
Regular nut product intake, n (%)	1452 (36.6)	1062 (37.5)
Children's characteristics		
Female, n (%)	1911 (48.2)	1380 (48.7)
Birth weight (g), mean, (SD)	3507.2 (546.1)	3526.0 (533.92)
Older siblings present, n (%)	1994 (50.3)	1414 (49.9)
Ever breast fed, n (%)	3200 (80.8)	2365 (83.5)

*Participants with data on pregnancy questionnaire, at least one of the outcome time points and all confounders.

†Assessed by validated screening questionaire.

‡Vitamin A/D, vitamin B, vitamin C, multivitamin, folic acid, calcium, iron or other vitamins.

§At age 3 months.

¶Reported 1 yr after pregnancy.

Table 4.2: *Maternal and child's characteristics of the respondents at baseline, those at follow–up 8 years, and those with IgE data at 8 years of age*

Maternal characteristic	**Respondents at baseline** **N=3963**	**Respondents at 8 yr** **N=3320**	**Respondents with IgE data at 8 yr** **N=1657**
Atopy ever, n (%)	1237 (31.2)	959 (28.9)*	625 (37.7)*
Partner ever atopic, n (%)	1217 (30.7)	1017 (30.6)	530 (32.0)
Asthma ever, n (%)	314 (7.9)	232 (7.0)*	152 (9.2)
Partner ever asthmatic, n (%)	302 (7.6)	245 (7.4)	140 (8.4)
Educational level low, n (%)	894 (22.6)	713 (21.5)*	338 (20.4)
Educational level high, n (%)	1331 (33.6)	1206 (36.3)*	629 (38.0)
Partner's educational level low, n (%)	973 (24.6)	808 (24.3)*	375 (22.6)
Partner's educational level high, n (%)	1493 (37.7)	1348 (40.6)*	698 (42.1)
Smoking during pregnancy, n (%)	696 (17.6)	523 (15.8)*	249 (15.0)
Supplement use during pregnancy, n (%)	3285 (82.9)	2761 (83.2)	1379 (83.2)
Smoking in the house by mother, father or others at baseline, n (%)	970 (24.5)	741 (18.7)*	356 (21.5)
Region north, n (%)	1231 (31.1)	1049 (31.6)	439 (26.5)*
Region central, n (%)	1586 (40.0)	1368 (41.2)	762 (46.0)
Region south-west, n (%)	1146 (28.9)	903 (27.2)*	456 (27.5)
Part in intervention study, n (%)	781 (19.7)	571 (17.2)*	398 (24.0)*
Age when having child, mean (SD)	30.3 (3.9)	30.6 (3.8)*	30.7 (3.8)*
Body Mass Index (kg/m^2), mean (SD)	23.3 (3.6)	23.3 (3.6)	23.4 (3.6)
Food intake during pregnancy			
Daily vegetable intake, n (%)	2217 (55.9)	1882 (56.7)	953 (57.5)
Daily fruit intake, n (%)	3025 (76.3)	2572 (77.5)*	1313 (79.2)
Daily + regular fish intake, n (%)	973 (24.6)	800 (24.1)	411 (24.8)
Daily + regular egg intake, n (%)	2631 (66.4)	2231 (67.2)	1127 (68.0)
Daily dairy intake, n (%)	3335 (84.2)	2840 (85.5)*	1434 (86.5)
Daily + regular nuts intake, n (%)	1322 (33.4)	1131 (34.1)	554 (33.4)
Daily nut product intake, n (%)	243 (6.1)	210 (6.3)	88 (5.3)
Regular nut product intake, n (%)	1452 (36.6)	1219 (36.7)	618 (37.3)
Children's characteristics			
Female, n (%)	1911 (48.2)	1615 (48.6)	799 (48.2)
Birth weight (g), mean (SD)	3507.2 (546.1)	3524.4 (534.8)*	3533.5 (521.2)
Older siblings present, n (%)	1994 (50.3)	1670 (50.3)	863 (52.1)
Ever breast fed, n (%)	3200 (80.8)	2734 (82.4)*	1392 (82.8)

*$p<0.01$: responders at 8 yr or responders with IgE data at 8 yr vs non-responders at 8 yr or responders at 8 yr without IgE data at 8 yr respectively.

Table 4.3: *Frequency of maternal food consumption during pregnancy*

	Consumption frequency (N=3963)		
Food group	**Rarely*** **n (%)**	**Regularly†** **n (%)**	**Daily‡** **n (%)**
Vegetables	29 (0.7)	1700 (42.9)	2217 (55.9)
Fruit	64 (1.6)	853 (21.5)	3025 (76.3)
Fish	2949 (74.4)	972 (24.5)	1 (0.0)
Egg	1296 (32.7)	2625 (66.2)	6 (0.2)
Dairy	52 (1.3)	476 (12.0)	3335 (84.2)
Nuts	2587 (65.3)	1267 (32.0)	55 (1.4)
Nut products	2216 (55.9)	1452 (36.6)	243 (6.1)

*Never or 1 to 3 times a month.
†Once or more than 4 times a week.
‡Once a day or more.

Table 4.4: *Prevalence of childhood symptoms of asthma from 1 to 8 years of age*

	Age							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Symptoms	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Wheeze	780 (20.4)	668 (17.9)	578 (15.7)	421 (11.8)	334 (9.5)	271 (7.8)	198 (5.9)	221 (6.7)
Wheeze without cold	-	-	182 (4.9)	150 (4.2)	136 (3.9)	132 (3.8)	108 (3.2)	115 (3.5)
Dyspnea	-	-	564 (15.3)	466 (13.1)	436 (12.4)	370 (10.7)	258 (7.7)	303 (9.1)
Dyspnea without cold	-	-	217 (5.9)	179 (5.0)	208 (5.9)	188 (5.4)	146 (4.3)	173 (5.2)
Steroid use	-	-	287 (7.8)	273 (7.7)	278 (7.9)	263 (7.6)	229 (6.8)	211 (6.4)
Asthma symptoms*	-	-	854 (23.1)	676 (19.0)	624 (17.7)	515 (14.8)	419 (12.4)	437 (13.2)
Doctor-diagnosed asthma	-	-	154 (4.2)	147 (4.1)	139 (4.0)	134 (3.9)	95 (2.8)	119 (3.9)

*Composite variable of wheeze, dyspnea or steroid use.

Table 4.5: *Overall associations of maternal food consumption during pregnancy and childhood asthma outcomes from 1 to 8 years of age*

		Wheeze	Dyspnea	Steroid use	Asthma symptoms*
Food consumption	**N**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Vegetables (daily vs regular+rare)					
Univariate	2830	0.97 (0.85–1.12)	1.01 (0.86–1.19)	0.93 (0.75-1.15)	0.98 (0.85-1.13)
Multivariate†	2830	0.97 (0.83-1.12)	0.99 (0.84-1.17)	0.96 (0.76-1.20)	0.98 (0.84-1.14)
Fruit (daily vs regular+rare)					
Univariate	2828	0.82 (0.70-0.96)	0.87 (0.72-1.06)	0.84 (0.65-1.09)	0.87 (0.73-1.04)
Multivariate†	2828	0.89 (0.75-1.04)	0.90 (0.74-1.10)	0.89 (0.68-1.16)	0.91 (0.77-1.09)
Fish (daily+regular vs rare)					
Univariate	2811	1.15 (0.99-1.35)	1.11 (0.92-1.33)	0.86 (0.67-1.12)	1.03 (0.88-1.23)
Multivariate†	2811	1.10 (0.94-1.29)	1.07 (0.89-1.29)	0.85 (0.66-1.10)	1.01 (0.85-1.20)
Egg (daily+regular vs rare)					
Univariate	2818	0.97 (0.84-1.12)	1.10 (0.92-1.31)	0.99 (0.79-1.25)	1.02 (0.87-1.19)
Multivariate†	2818	0.96 (0.84-1.12)	1.12 (0.94-1.34)	1.01 (0.80-1.28)	1.03 (0.88-1.20)
Dairy (daily vs regular+rare)					
Univariate	2788	0.84 (0.68-1.03)	0.90 (0.71-1.16)	0.99 (0.72-1.36)	0.89 (0.72-1.10)
Multivariate†	2788	0.88 (0.71-1.09)	0.92 (0.72-1.19)	1.03 (0.74-1.43)	0.92 (0.74-1.15)
Nuts (daily+regular vs rare)					
Univariate	2806	0.96 (0.83-1.12)	1.00 (0.82-1.19)	0.95 (0.75-1.19)	0.96 (0.82-1.11)
Multivariate†	2806	0.99 (0.86-1.15)	1.04 (0.88-1.23)	1.03 (0.81-1.29)	1.00 (0.86-1.17)
Nut products (regular vs rare)					
Univariate	2812	1.04 (0.90-1.21)	0.99 (0.84-1.18)	0.94 (0.74-1.18)	0.99 (0.85-1.15)
Multivariate†	2812	1.01 (0.88-1.18)	0.98 (0.82-1.16)	0.94 (0.74-1.19)	0.98 (0.84-1.14)
Nut products (daily vs rare)					
Univariate	2812	1.39 (1.05-1.86)	1.52 (1.12-2.06)	1.48 (0.98-2.22)	1.41 (1.05-1.89)
Multivariate†	2812	1.42 (1.06-1.89)	1.58 (1.16-2.15)	1.62 (1.06-2.46)	1.47 (1.08-1.99)

*Composite variable of wheeze, dyspnea or steroid use.

†Adjusted for sex, maternal educational level, parental atopy, maternal smoking during pregnancy, smoking in the house at age 8 years, breast feeding, presence of older siblings, birth weight, overweight mother, maternal supplement use during pregnancy without folic acid and iron, region and study arm (intervention study or natural history study).

Children of mothers who daily ate fresh fruit had a crude decreased risk of wheeze versus children of mothers who did not eat fruit on a daily basis. However, this changed to borderline significant after adjustment for confounders in the multivariate analysis. There were no significant overall associations between fruit consumption and the other childhood outcomes. Figure 4.1 shows the course of the associations between maternal fruit consumption during pregnancy and childhood outcomes over time. These plots show that the significance of the crude overall association between fruit and wheeze is strongest determined by the (borderline) significant associations at 1, 2 and 5 years of age. Furthermore, we can see occasional significant associations at certain ages, whereas overall associations are non-significant (e.g. for fruit and steroid use at age 6).

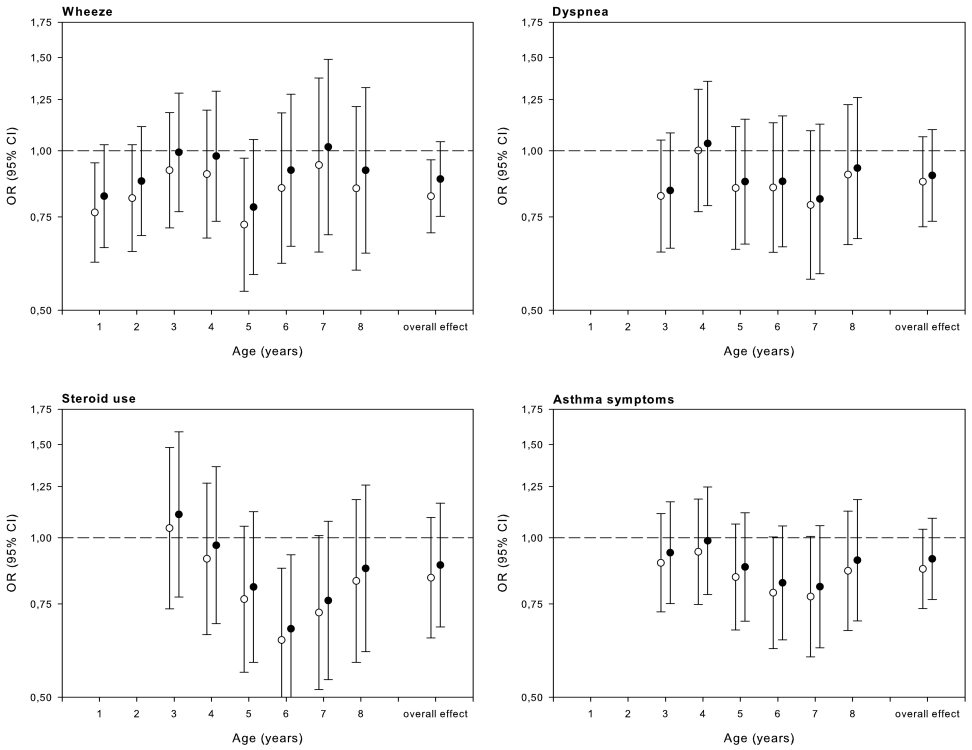

Figure 4.1: *Odds ratios with 95% confidence intervals of the crude (∘) and adjusted (•) associations of daily versus regular and rare fruit consumption during pregnancy and childhood wheeze, dyspnea, steroid use and asthma symptoms from 1 to 8 years of age*

Daily consumption versus rare consumption of nut products during pregnancy was significantly positively associated with childhood wheeze, dyspnea, steroid use and 'asthma symptoms'. Regular consumption versus rare consumption of nut products

during pregnancy was not associated with any of the outcomes. P–values for trend (daily — regular — rare consumption) were not statistically significant (data not shown). Adjustment for potential confounders and stratification by maternal allergy did not consistently alter the magnitude or significance of the results. Plots of the course of the associations between maternal daily nut product consumption versus rare nut product consumption during pregnancy and childhood symptoms of asthma over time are shown in figure 4.2. These plots show that the adverse associations are consistent over time.

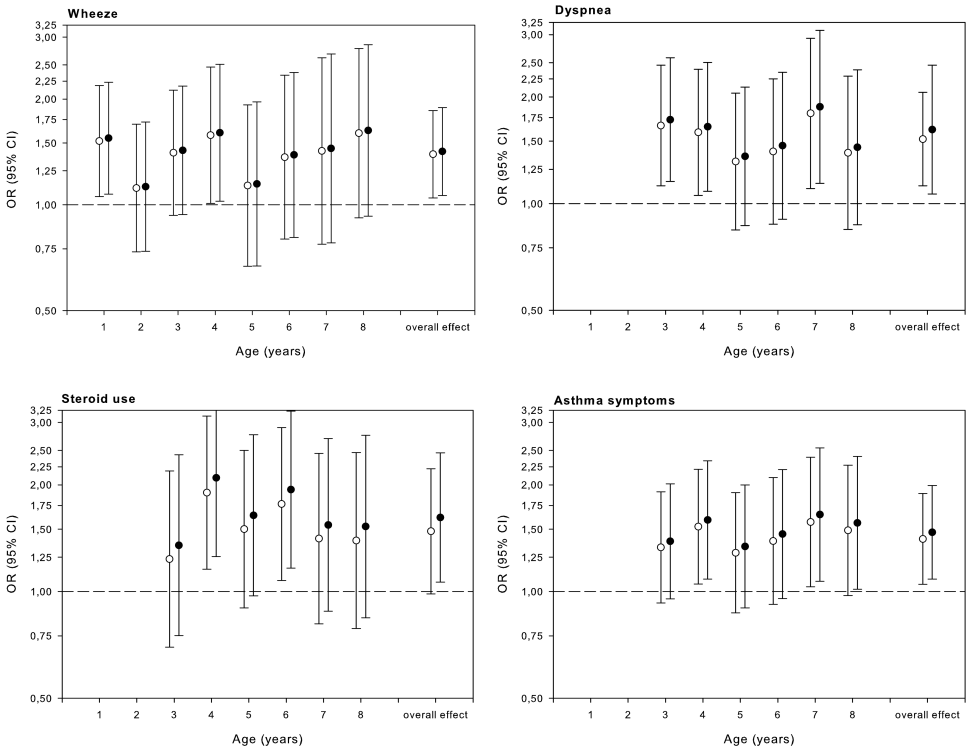

Figure 4.2: *Odds ratios with 95% confidence intervals of the crude (∘) and adjusted (•) associations of daily versus rare nut product consumption during pregnancy and childhood wheeze, dyspnea, steroid use and asthma symptoms from 1 to 8 years of age*

Table 4.6 shows additional results of the overall associations of the different food groups with wheeze without cold, dyspnea without cold and doctor-diagnosed asthma from 3 to 8 years of age. Daily versus rare nut products consumption during pregnancy was strongly positively associated with these additional childhood outcomes as well.

Table 4.6: *Overall associations of maternal food consumption during pregnancy and additional childhood asthma outcomes from 1 to 8 years of age*

		Wheeze without cold	Dyspnea without cold	Doctor-diagnosed asthma
Food consumption	**N**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Vegetables (daily vs regular+rare)				
Univariate	2830	0.96 (0.74-1.23)	1.01 (0.81-1.26)	1.04 (0.82-1.32)
Multivariate*	2830	0.99 (0.76-1.29)	1.00 (0.80-1.26)	1.00 (0.77-1.29)
Fruit (daily vs regular+rare)				
Univariate	2828	0.84 (0.63-1.13)	0.84 (0.65-1.10)	0.95 (0.72-1.26)
Multivariate*	2828	0.92 (0.69-1.24)	0.87 (0.67-1.15)	0.99 (0.75-1.32)
Fish (daily+regular vs rare)				
Univariate	2811	1.16 (0.87-1.55)	1.14 (0.89-1.46)	1.19 (0.90-1.57)
Multivariate*	2811	1.10 (0.83-1.47)	1.09 (0.85-1.39)	1.11 (0.84-1.48)
Egg (daily+regular vs rare)				
Univariate	2818	1.02 (0.78-1.35)	1.04 (0.82-1.33)	1.19 (0.91-1.55)
Multivariate*	2818	1.05 (0.80-1.38)	1.06 (0.83-1.36)	1.19 (0.91-1.55)
Dairy (daily vs regular+rare)				
Univariate	2788	0.74 (0.52-1.04)	0.81 (0.59-1.12)	1.12 (0.75-1.65)
Multivariate*	2788	0.78 (0.54-1.11)	0.84 (0.60-1.17)	1.11 (0.75-1.66)
Nuts (daily+regular vs rare)				
Univariate	2806	0.93 (0.72-1.21)	0.96 (0.76-1.20)	0.99 (0.77-1.28)
Multivariate*	2806	1.00 (0.77-1.30)	1.02 (0.81-1.28)	1.06 (0.82-1.36)
Nut products (regular vs rare)				
Univariate	2812	0.98 (0.74-1.28)	0.91 (0.72-1.15)	1.10 (0.86-1.42)
Multivariate*	2812	0.96 (0.73-1.26)	0.89 (0.70-1.13)	1.08 (0.83-2.60)
Nut products (daily vs rare)				
Univariate	2812	1.71 (1.08-2.69)	1.76 (1.22-2.56)	1.49 (0.96-2.33)
Multivariate*	2812	1.84 (1.16-2.91)	1.89 (1.30-2.75)	1.64 (1.03-2.60)

*Adjusted for sex, maternal educational level, parental atopy, maternal smoking during pregnancy, smoking in the house at age 8 years, breast feeding, presence of older siblings, birth weight, overweight mother, maternal supplement use during pregnancy without folic acid and iron, region and study arm (intervention study or natural history study).

Table 4.7: *Associations of maternal food consumption during pregnancy and wheezing patterns in children from 1 to 8 years of age*

		Early transient wheeze*	Late–onset wheeze†	Persistent wheeze‡
Food consumption	**N**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Vegetables (daily vs regular+rare)				
Univariate	2830	0.85 (0.71-1.02)	0.78 (0.53-1.15)	1.07 (0.81-1.41)
Multivariate§	2830	0.82 (0.67-0.99)	0.78 (0.53-1.17)	1.07 (0.79-1.44)
Fruit (daily vs regular+rare)				
Univariate	2828	0.74 (0.59-0.92)	0.63 (0.41-0.96)	0.86 (0.61-1.19)
Multivariate§	2828	0.80 (0.63-1.00)	0.62 (0.40-0.96)	0.95 (0.67-1.34)
Fish (daily+regular vs rare)				
Univariate	2811	1.21 (0.98-1.50)	0.77 (0.48-1.25)	1.24 (0.91-1.69)
Multivariate§	2811	1.16 (0.93-1.43)	0.71 (0.44-1.15)	1.16 (0.84-1.60)
Egg (daily+regular vs rare)				
Univariate	2818	0.96 (0.79-1.16)	0.88 (0.60-1.31)	0.93 (0.70-1.24)
Multivariate§	2818	0.96 (0.78-1.17)	0.89 (0.60-1.33)	0.93 (0.69-1.25)
Dairy (daily vs regular+rare)				
Univariate	2788	0.92 (0.69-1.22)	0.65 (0.39-1.09)	0.69 (0.47-1.02)
Multivariate§	2788	0.98 (0.73-1.32)	0.68 (0.40-1.15)	0.73 (0.48-1.09)
Nuts (daily+regular vs rare)				
Univariate	2806	0.95 (0.79-1.16)	0.89 (0.59-1.33)	0.94 (0.70-1.25)
Multivariate§	2806	0.97 (0.79-1.18)	0.92 (0.61-1.38)	1.01 (0.75-1.36)
Nut products (regular vs rare)				
Univariate	2812	1.01 (0.83-1.22)	1.08 (0.73-1.60)	1.01 (0.75-1.35)
Multivariate§	2812	0.98 (0.80-1.19)	1.11 (0.75-1.65)	1.00 (0.74-1.36)
Nut products (daily vs rare)				
Univariate	2812	1.09 (0.74-1.62)	0.94 (0.40-2.23)	1.84 (1.12-3.02)
Multivariate§	2812	1.08 (0.72-1.61)	1.03 (0.43-2.46)	2.14 (1.29-3.56)

*Wheeze during the first three years of life but not from 6 to 8 years of age.

†No wheeze during first three years of life but wheeze from 6 to 8 years of age.

‡Wheeze during the first three years of life and from 6 to 8 years of age as well.

§Adjusted for sex, maternal educational level, parental atopy, maternal smoking during pregnancy, smoking in the house at 8 years of age, breast feeding, presence of older siblings, birth weight, overweight mother, maternal supplement use during pregnancy without folic acid and iron, region and study arm (intervention study or natural history study).

Results of the analyses of maternal food consumption during pregnancy and the different wheezing phenotypes at 8 years of age show a strong positive association between daily nut product consumption and persistent wheeze, which is in line with the consistently positive associations over the entire time period as shown by the GEE analyses (table 4.7).

4.3.3 Sensitivity analyses

IgE data was present for a subgroup at age 8. We investigated if maternal nut product consumption during pregnancy was also associated with sensitisation to food allergens (one or more specific IgE $\geq$0.35 IU/ml to milk or egg), sensitisation to inhalant allergens (one or more specific IgE $\geq$0.35 IU/ml to house dust mite, cat, dog, dactylis, birch or alternaria) or a high total IgE level (total IgE $\geq$100 IU/ml) in these children. This was not the case.

Maternal diet during pregnancy and the child's diet at age 2 were strongly associated. However, including the child's consumption of fruit, vegetables, fish, egg, and peanut butter at age 2 in the models did not change the results. Neither did inclusion of the child's full-fat milk and butter consumption, which have been associated with doctor-diagnosed asthma and wheeze in earlier cross-sectional analyses of the same cohort.[30]

Maternal (food) allergy could by a reason to avoid consumption of certain foods, and therefore bias the results. We have compared the dietary intake of atopic and non-atopic mothers and found that there were no significant differences in frequencies of consumption of the investigated food groups. Exclusion of mothers who never ate fish, egg or nuts and nut products during the last month from the analyses did not change the results. We also checked whether mothers who daily consumed nut products during pregnancy differed from mothers who regularly or rarely consumed nut products, regarding characteristics listed in table 4.1. This was not the case except that the daily consumers were more likely to be from region north.

Food consumption frequencies were initially classified into easily interpretable categories: rare, regular and daily consumption. These categories were condensed if one of them contained less than 5% of the observations, which made some of them quite large. We have checked whether a different classification of the food groups into more categories changed the results. The results of these additional analyses were very similar to the previous results when using the more condensed classification.

Supplements were used by almost 83% of the pregnant women. About 50% used folic acid and/or iron, whereas the use of the other supplements ranged from about 3% for vitamin A/D to about 16% for the use of other supplements, which were mostly multivitamins for pregnant women. We checked if consumption of a certain amount of the specific foods was associated with use of certain supplements. Positive associations were seen between daily fruit, vegetable and milk product consumption and folic acid, regular plus daily fish consumption and vitamin B and C, and regular plus daily nuts consumption and vitamin A/D, vitamin B, multivitamin, folic acid and calcium. Negative associations were seen between daily fruit consumption and vitamin C and iron, daily milk consumption and calcium and iron, and daily milk

products consumption and iron. All multivariate analyses were adjusted for maternal supplement use, however, this did not alter the results.

4.4 Discussion

We have investigated the associations between maternal consumption of fresh fruit, vegetables, fish, egg, dairy, nuts and nut products such as peanut butter during pregnancy and childhood symptoms of asthma longitudinally from 1 to 8 years of age. Our results showed no consistent associations between the maternal intake of the investigated food groups during pregnancy and childhood asthma symptoms until the age of 8, except for nut products. Daily versus rare nut products consumption during pregnancy was consistently significantly positively associated with childhood wheeze (without cold), dyspnea (without cold), steroid use, doctor-diagnosed asthma and the composite variable 'asthma symptoms'.

An important issue when studying the effect of dietary intake on asthma or allergy is whether the effects we find can be attributed to specific nutrients, specific foods, or to aspects of a 'healthy' diet or lifestyle.[31] In this study the crude association between maternal fruit intake during pregnancy and childhood wheeze lost statistical significance after adjustment for maternal education, maternal smoking during pregnancy, smoking in the home at 8 years of age, breast feeding, presence of older siblings, birth weight, overweight of the mother and region. This suggests that there may be confounding by socioeconomic and lifestyle factors. The Dutch national food consumption surveys carried out in the last two decades have all confirmed that socioeconomic status (based on education, occupation and occupational position), and factors like region and alcohol consumption were associated with fruit, fruit juice and vegetable consumption.[32,33] Besides confounding by socioeconomic and lifestyle factors there is the issue of health consciousness. Daily fruit consumption during pregnancy was associated with the use of folic acid, which might be an indication that mothers who daily eat fruit are more health conscious and might be more likely to report symptoms in their child. This would lead to an increased risk of childhood wheeze, which is not the case in our study.

A limitation of the present study is that we only had information on the frequency of intake of larger food groups. The questionnaire did not contain questions on intake of more specific foods or portion sizes. The limited distribution of the consumption frequency of large food groups makes it difficult to detect effects. The SEATON study conducted in the UK reported beneficial effects of maternal apple consumption during pregnancy on wheeze and asthma in 5-year-olds, but not of maternal total fruit consumption.[22] This suggests that there may be effects of fruit specific nutrients which can not be found when only analysing the effect of total fruit consumption. In general, apples, tangerines and bananas are the most consumed fruits in the Netherlands.[33,34] The food questions in the pregnancy questionnaire were not validated against other dietary instruments or biomarkers. Although the main food groups associated with asthma were included in our questionnaire confounding by other foods or nutrients cannot be completely ruled out. Dietary misreporting could have lead to dietary

misclassification, however, that seems unlikely to have happened non-randomly.

We did not find an effect of maternal fish consumption during pregnancy on childhood asthma outcomes. A number of studies have suggested that higher fish intake in pregnancy might be protective for asthma or allergy.[18–23] Yet, the current evidence is inconclusive. For instance, the SEATON study found a protective effect of higher fish consumption during pregnancy on eczema but not on wheeze or asthma,[22] whereas the study of Salam and colleagues[18] only found an effect in children of asthmatic mothers. The effects of childhood fish consumption on childhood asthma/allergy are inconsistent as well.[1,31]

This study shows a consistent increased risk of daily consumption of nut products during pregnancy on childhood asthma outcomes. Because we literally mentioned peanut butter as an example of nut products in the questionnaire, and because peanut butter is a commonly used spread on sandwiches in the Netherlands, whereas the use of other nut spreads is not very common, we assumed that the largest proportion of nut products is peanut butter. The observed positive associations might thus be due to a high consumption of peanut butter during pregnancy. One possible explanation could be that a higher exposure to peanut allergens in foetuses of mothers who daily consume peanut butter during pregnancy might increase their risk of developing symptoms of asthma. The study of Sausenthaler and colleagues[20] also suggested that intake of allergenic foods during pregnancy may increase the risk of allergic disease in the offspring. Higher intake of celery and citrus fruit during pregnancy increased the risk of sensitisation against food allergens (egg, cow's milk, wheat, peanut, soybean or codfish), and higher intake of raw sweet peppers and citrus fruit was associated with sensitisation against inhalant allergens. Antenatal exposure to allergens can occur through diaplacental transport[35] and transamniotic transfer.[36] This may stimulate the fetal immune system leading to sensitisation,[9] which might increase the risk of developing atopic disease. However, we did not find associations between daily nut product consumption during pregnancy and sensitisation to food (milk, egg) or inhalant allergens at 8 years of age. Data on sensitisation to peanut allergens was not available in our study, but we did check for an association with reported peanut allergy. Daily nut product consumption was not associated with 'ever having doctor-diagnosed peanut allergy' reported at age 8.

Studies that have investigated the effect of maternal allergen avoidance during pregnancy on sensitisation and atopic disease in children provide conflicting results. Adherence to a low allergen content diet during pregnancy and lactation is currently considered as potentially harmful for possible maternal and fetal malnutrition and is not recommended.[10] There might be an exception for peanut. Peanut is a very potent allergen and peanut allergy is associated with anaphylaxis and less likely to be outgrown than for instance cow's milk or egg allergy.[37] Studies of Hourihane an colleagues[38] and Frank and colleagues[39] found that higher peanut consumption during pregnancy and early introduction of peanut to the child's diet was associated with more peanut allergy, and that peanut allergy was associated with asthma, eczema and rhinitis in children. Since 1998, the UK government recommends pregnant women with a (family) history of atopic disease to avoid peanuts during pregnancy or breast feeding, and that infants should not be exposed until 3 years of age. Hourihane and

colleagues[40] investigated the prevalence of peanut allergy in children at school entry from 2003 to 2005, but found no effect of this advice. In the Netherlands there is no recommendation to avoid peanuts during pregnancy or lactation.

Another explanation for the increased risk of daily nut product consumption during pregnancy on childhood asthma symptoms could be increased intake of linoleic acid. Peanut butter contains approximately 20% linoleic acid.[41] Increased intake of linoleic acid may lead to higher levels of cellular arachidonic acid, which in turn increases the capacity to produce prostaglandin E2 (PGE2). There is evidence that PGE2 can alter the balance of Th1 and Th2 cytokines leading to increased formation of IgE, and hence atopic disease.[42,43] The average intake of linoleic acid by Dutch pregnant women in 1998 was 12 g/day.[44] Ten grams of peanut butter on a sandwich contains approximately 2 grams of linoleic acid,[41] so daily consumption of one or two peanut butter sandwiches may substantially increase the intake of linoleic acid. Unfortunately, we did not have data on the consumption of other N6-rich foods during pregnancy such as margarine.

Nuts are a source of vitamin E; the Dutch obtain 6% of their vitamin E intake through consumption of nuts and snacks.[34] Daily nut product consumption might thus increase vitamin E intake, which has been found protective for wheeze and asthma in the SEATON study. However, these protective effects were mainly seen for the highest quintile of vitamin E intake (9.17-30.8 mg/day).[13] This level is not necessarily achieved by daily consumption of, for instance a peanut butter sandwich (10 g peanut butter contains 0.97 mg vitamin E).[41] This study adds to the evidence of effects of fetal life exposure on childhood asthma and allergy. A strong point to our opinion is that the longitudinal analysis method made it possible to study the development of associations between maternal diet during pregnancy and childhood asthma symptoms over time. Cross-sectional associations between maternal diet during pregnancy and childhood symptoms might be incidental findings. Longitudinal analyses are important to reveal temporality of effects, and to strengthen the evidence of causal relationships between maternal diet during pregnancy and childhood allergic disease. With GEE the relationships between the variables of the model at different time points are analysed simultaneously, which makes chance findings because of multiple testing less likely than when using logistic regression analyses for all the outcomes at different time points separately. After 8 years of follow-up around 80% of the population initially recruited was still participating. Like in other prospective studies, participants with a lower socioeconomic status, a less healthy lifestyle and poorer health were more likely to become lost to follow-up than others.[30] This type of response bias can possibly result in underestimated effects of daily fruit consumption, as a larger proportion of symptomatic children with mothers who do not daily eat fruit would make the associations stronger. However, in our study the proportion of mothers who daily ate fruit in the baseline population was similar to the proportion in the population who provided data at 8 years of age (table 4.2).

Maternal diet during pregnancy was strongly associated with the child's diet. However, the observed associations between maternal diet during pregnancy and childhood asthma outcomes were independent from the child's diet. Including the child's diet in the models did not change the results.

The results of this study indicate a small beneficial effect of daily fruit consumption on wheeze (borderline significant after adjustment for potential confounding factors) and an increased risk of daily nut product consumption during pregnancy on childhood symptoms of asthma. Future studies need to unravel if beneficial effects of maternal fruit consumption during pregnancy can be attributed to specific nutrients, specific kinds of fruit, or that fruit consumption is an indicator of a healthier life-style. This can be done by using more extensive food frequency questionnaires to better assess portion sizes and types of fruit that participants consume. More research is needed to study the effect of exposure to nut or nut products or other allergenic foods during pregnancy, not only on the development of food allergy, but also on the development of asthma and other allergic diseases. The findings of this study need to be replicated by other studies before giving dietary advice to pregnant women.

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Chapter 5

Tracking of childhood food consumption: the PIAMA study

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Abstract

Background Several studies have provided evidence on the relation between nutrition and health outcomes in children. However, nutrition is usually just measured once in time, and it is not clear how well measurements at one point in time reflect nutrition over a longer period.

Objectives The aim of this study was to assess the stability of dietary habits during childhood.

Methods The PIAMA birth cohort consisted of a baseline population of 4146 participants which were followed until 8 years of age. Annual questionnaires provided longitudinal data on the frequency of intake of a number of foods and food groups. Tracking models based on generalised estimating equations (GEE) were used to describe stability of dietary habits over time.

Main results Daily fruit and vegetable consumption decreased considerably over time. Highest tracking was observed for butter consumption. Tracking of fruit, cooked vegetables, fish, margarine and semi-skimmed milk consumption were moderate to low. Tracking of fruit, fish and margarine consumption significantly differed by maternal educational level.

Conclusion The assumption that dietary consumption estimated at a certain time point reflects the habitual intake during childhood is not valid for all foods. Childhood diet changes over time, so longitudinal studies in children can provide better estimates of dietary exposure, and can be used to assess effects of changes in diet on children's health.

5.1 Introduction

Several studies have provided evidence on the relation between nutrition and health outcomes in children. However, nutrition is usually just measured cross-sectionally, and it is not clear how well measurements at one point in time reflect nutrition over a longer period. Our longitudinal birth cohort has annually assessed dietary habits until 8 years of age. This information can be used to investigate the development of dietary habits from early to later age, which can be done by tracking analyses. Tracking can be defined as the stability of a certain determinant over time, as well as the predictability of a measurement of a certain determinant early in life for values of the same determinant later in life.[1] The objectives of the current study were to investigate changes of dietary habits over time and, the tracking of the consumption of different foods, that is, how strongly the frequency of intake of certain foods at a specific age is associated with intakes at earlier and later ages. If dietary habits 'track' very well, then dietary exposure measured at some point in time is possibly valid for a longer period during childhood, which makes the assessment of associations with health outcomes less complex. In addition, this information can be used to guide early intervention strategies to promote healthy eating in children.

5.2 Methods

5.2.1 Study population and study design

The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study was set up in 1996 to study different determinants for asthma and allergic disease in children. Details of the PIAMA study design have been published previously.[2] The baseline population consisted of 4146 pregnant women, 183 participants were lost to follow-up before any data on the child had been collected, therefore the study started with 3963 newborn children. Children were born between summer 1996 and late fall 1997, and follow-up by questionnaire took place when they were 3 months of age, and annually from 1 to 8 years of age.

5.2.2 Assessment of childhood food consumption

The annual questionnaires enquired about the frequency of consumption of approximately 30 to 35 different foods or food groups by the child in the preceding month. For the present study we were interested in the child's consumption of foods and food groups high in antioxidants (fruit, vegetables and brown/wholemeal bread), n-3 fatty acids (fish), n-6 fatty acids (margarine) and milk fat (full cream milk, semi-skimmed milk and butter) from 2 to 8 years of age. Answer options for the frequency of consumption in the last months were: (1) never, (2) less than once a week, (3) one to two days a week, (4) three to five days a week, and (5) six to seven days a week. These answer options were grouped into three categories: rare consumption (option 1 and 2), regular consumption (option 3 and 4) and daily consumption (option 5). Dichotomous variables of food intake levels were used for the longitudinal tracking

analyses. For fresh fruit, cooked vegetables, brown/wholemeal bread, full cream milk, semi-skimmed milk, butter and margarine, high versus low consumption was defined as daily consumption versus regular plus rare consumption. For fish, high versus low consumption was defined as daily plus regular consumption versus rare consumption. Long-term high consumption was defined as high consumption at all ages.

5.2.3 Statistical analyses

All statistical analyses were carried out using SAS for Windows version 9.1 (SAS Institute, Cary, NC, USA). Generalised Estimating Equations (GEE) can be used to analyze tracking when there are more than two repeated measurements.[3] Logistic GEE was used to estimate the odds for being in the high consumption category during follow-up (e.g. from age 3 to 8) for children that were in this category at the initial measurement (e.g. age 2), compared to children that were in the low consumption category at the initial measurement. The resulting coefficient $\beta 1$ reflects this relationship and can be transformed into an odds ratio.[3] The GEE model has been described in the appendix (equation 5.1). All analyses were adjusted for potential confounders: sex, maternal education, parental atopy, allergic sibling, maternal smoking during pregnancy, smoking in the house at 3 months of age, breastfeeding, birth weight, maternal overweight (maternal BMI $\geq$25 kg/m^2 assessed at 1 year follow-up) and region (north, central, south-west). An interaction term between the predictor variable and maternal educational level was added to the models to check for effect-modification.

5.3 Results

5.3.1 Study population

Questionnaire data from age 1 to 8 years were obtained for 3817, 3740, 3694, 3563, 3518, 3473, 3373, and 3320 children respectively. Characteristics of the study population at baseline (N=3963) and of the participants with complete dietary data from 2 to 8 years of age for at least one of the investigated food groups (N=2870) are described in table 5.1.

Children with complete dietary data were more likely to have a mother with a high educational level, to have been breast fed, and to have a higher mean birth weight compared to children who did not have complete data. Furthermore, they were less likely to have a mother with a low educational level, a mother who smoked during pregnancy or to have been exposed to smoking in the home at baseline, and to be from region south-west. Yet, the magnitude of the differences between both populations was small.

Table 5.1: *Characteristics of the study population at baseline and complete cases*

Characteristics	**Respondents at baseline N=3963 n (%)**	**Complete cases*** **N=2870 n (%)**
Female child	1911 (48.2)	1393 (48.5)
Atopic mother	821 (20.7)	516 (18.0†)
Atopic father	801 (20.2)	611 (21.3†)
Both parents atopic	416 (10.5)	251 (8.8†)
Allergic sibling	756 (19.2)	539 (18.8)
Maternal education low	894 (23.5)	596 (20.8†)
Maternal education intermediate	1582 (41.5)	1219 (42.5)
Maternal education high	1331 (35.0)	1051 (36.7†)
Maternal smoking during pregnancy	696 (17.8)	433 (15.3†)
Smoking in the home at baseline	970 (24.5)	613 (21.5†)
Breastfeeding ever	3200 (82.1)	2374 (83.4†)
Birth weight low ($\leq$3000 g)	353 (13.8)	351 (12.3†)
Birth weight normal (3001-3999 g)	2677 (68.2)	1970 (68.8)
Birth weight high ($\geq$4000 g)	702 (18.0)	544 (19.0†)
Maternal overweight (BMI $\geq$25 kg/m^2)	886 (25.1)	671 (24.9)
Region north	1231 (31.1)	911 (31.7)
Region central	1586 (40.0)	1212 (42.2)
Region south-west	1146 (28.9)	747 (26.0†)

*Participants with all dietary data for at least one of the investigated food groups from age 2 until age 8.

†$p<0.01$: complete cases included in the analyses vs non-complete cases

Table 5.2: *Consumption frequencies for selected foods and food groups from 2 to 8 years of age*

Food	N*	Frequency	Age 2 n (%)	Age 3 n (%)	Age 4 n (%)	Age 5 n (%)	Age 6 n (%)	Age 7 n (%)	Age 8 n (%)
Fresh fruit	2815	rarely	103 (3.6)	91 (3.2)	74 (2.6)	92 (3.4)	91 (3.2)	86 (3.1)	99 (3.5)
		regularly	795 (28.4)	959 (34.1)	974 (34.6)	1021 (36.3)	1030 (36.6)	1118 (39.7)	1081 (38.4)
		daily	1917 (68.1)	1765 (62.7)	1767 (62.8)	1702 (60.5)	1694 (60.2)	1611 (57.2)	1635 (58.1)
Cooked vegetables	2793	rarely	74 (2.6)	130 (4.6)	106 (3.8)	82 (2.9)	72 (2.6)	57 (2.0)	57 (2.0)
		regularly	1130 (40.5)	1387 (49.7)	1495 (53.5)	1692 (60.6)	1733 (62.0)	1713 (61.3)	1695 (60.7)
		daily	1589 (56.9)	1276 (45.7)	1192 (42.7)	1019 (36.5)	988 (35.4)	1023 (36.7)	1041 (37.3)
Brown/wholemeal bread	2839	rarely	87 (3.1)	91 (3.2)	95 (3.3)	118 (4.1)	113 (4.0)	106 (3.7)	117 (4.1)
		regularly	329 (11.6)	348 (12.3)	368 (13.0)	391 (13.8)	445 (15.7)	432 (15.2)	429 (15.1)
		daily	2423 (85.3)	2400 (84.5)	2376 (83.7)	2330 (82.1)	2281 (80.3)	2301 (81.1)	2293 (80.8)
Fish	2716	rarely	2094 (77.1)	1881 (69.3)	1789 (65.9)	1838 (67.7)	1728 (63.6)	1702 (62.7)	1685 (62.0)
		regularly	617 (22.7)	821 (30.2)	920 (33.9)	874 (32.2)	984 (36.2)	1009 (37.2)	1025 (37.7)
		daily	5 (0.2)	14 (0.5)	7 (0.3)	4 (0.2)	4 (0.2)	5 (0.2)	6 (0.2)
Full cream milk	2756	rarely	1720 (62.4)	2037 (73.9)	2203 (79.9)	2319 (84.1)	2431 (88.2)	2496 (90.6)	2550 (92.5)
		regularly	157 (5.7)	174 (6.3)	187 (6.8)	157 (5.7)	135 (4.9)	102 (3.7)	84 (3.1)
		daily	879 (31.9)	545 (19.8)	366 (13.3)	280 (10.2)	190 (6.9)	158 (5.7)	122 (4.4)
Semi-skimmed milk	2778	rarely	1014 (36.5)	860 (31.0)	744 (26.8)	729 (26.2)	752 (27.1)	770 (27.7)	766 (27.6)
		regularly	305 (11.0)	357 (12.8)	400 (14.4)	469 (16.9)	493 (17.7)	439 (15.8)	467 (16.8)
		daily	1459 (52.5)	1561 (56.2)	1634 (58.8)	1580 (56.9)	1533 (55.2)	1569 (56.5)	1545 (55.6)
Butter	2818	rarely	2471 (87.7)	2461 (87.3)	2457 (87.2)	2466 (88.2)	2489 (88.3)	2482 (88.1)	2501 (88.7)
		regularly	160 (5.7)	175 (6.2)	196 (6.9)	163 (5.8)	189 (6.7)	201 (7.1)	203 (7.2)
		daily	187 (6.6)	182 (6.5)	165 (5.9)	169 (6.0)	140 (5.0)	135 (4.8)	114 (4.1)
Margarine	2794	rarely	551 (19.7)	270 (9.7)	264 (9.5)	304 (10.9)	335 (12.0)	362 (13.0)	388 (13.9)
		regularly	418 (15.0)	321 (11.5)	320 (11.5)	342 (12.2)	341 (12.2)	372 (13.3)	417 (14.9)
		daily	1825 (65.3)	2203 (78.8)	2210 (79.1)	2148 (76.9)	2118 (75.8)	2060 (73.7)	1989 (71.2)

*Number of children with dietary information on that particular food from 2 to 8 years of age.

5.3.2 Dietary habits from 2 to 8 years of age

Table 5.2 shows the consumption frequency categories 'daily', 'regularly', and 'rarely' for fresh fruit, cooked vegetables, brown/wholemeal bread, fish, full cream milk, semi-skimmed milk, butter, and margarine consumption from 2 to 8 years of age.

Prevalence of long-term high consumption from 2 to 8 years of age is shown in table 5.3.

Table 5.4 shows tracking, that is, the odds of high fresh fruit, cooked vegetable, brown/wholemeal bread, fish, full cream milk, semi-skimmed milk, butter and margarine consumption from 3 to 8 years of age when being in the high consumption category at 2 years of age compared to children who were not in this category at 2 years of age.

Table 5.3: *Prevalence of long-term high consumption of the different food groups from 2 to 8 years of age*

Food	**N***	**Long-term high consumption n (%)**
Fresh fruit	2815	845 (30.0)
Cooked vegetables	2793	357 (12.8)
Brown/wholemeal bread	2839	1657 (59.8)
Fish	2716	183 (6.7)
Full cream milk	2756	59 (2.1)
Semi-skimmed milk	2778	688 (24.8)
Butter	2818	45 (1.6)
Margarine	2794	1192 (42.7)

*Number of children with dietary information on that particular food from 2 to 8 years of age.

Table 5.4: *Tracking odds ratios for being in the highest category of consumption during follow-up from 3 to 8 years of age*

Food	N*		Age 3 OR (95% CI)	Age 4 OR (95% CI)	Age 5 OR (95% CI)	Age 6 OR (95% CI)	Age 7 OR (95% CI)	Age 8 OR (95% CI)	Overall OR (95% CI)
Fresh fruit (daily)	3249	Crude	10.6 (8.9-12.5)	8.0 (6.8-9.5)	5.3 (4.5-6.2)	5.0 (4.2-5.8)	5.1 (4.3-6.0)	4.6 (3.9-5.4)	6.3 (5.6-7.1)
		Adjusted†	10.0 (8.4-11.9)	7.6 (6.4-9.0)	4.9 (4.2-5.8)	4.6 (3.9-5.5)	4.7 (4.0-5.6)	4.3 (3.6-5.0)	5.9 (5.2-6.6)
Cooked vegetables (daily)	3246	Crude	7.7 (6.6-9.1)	6.1 (5.2-7.2)	4.9 (4.2-5.9)	4.6 (4.0-5.6)	5.1 (4.3-6.1)	4.0 (3.4-4.7)	5.5 (4.9-6.2)
		Adjusted†	7.9 (6.7-9.4)	6.3 (5.3-7.4)	5.0 (4.2-6.0)	4.8 (4.1-5.7)	5.2 (4.4-6.2)	4.1 (3.4-4.8)	5.6 (5.0-6.3)
Brown/wholemeal bread (daily)	3251	Crude	14.4 (11.6-18.0)	11.2 (9.0-14.0)	8.1 (6.6-10.1)	6.8 (5.5-8.4)	6.0 (4.8-7.4)	5.9 (4.8-7.4)	8.4 (7.3-9.8)
		Adjusted†	12.9 (10.2-16.2)	9.9 (7.9-12.5)	7.0 (5.6-8.8)	5.9 (4.7-7.3)	5.1 (4.1-6.4)	5.1 (4.1-6.4)	7.3 (6.3-8.6)
Fish (daily + regular)	3218	Crude	7.9 (6.6-9.5)	5.5 (4.6-6.6)	4.6 (3.8-5.5)	5.1 (4.3-6.1)	4.7 (3.9-5.7)	3.8 (3.2-4.6)	5.2 (4.6-5.9)
		Adjusted†	7.6 (6.4-9.2)	5.3 (4.5-6.4)	4.4 (3.7-5.3)	4.9 (4.1-5.9)	4.5 (3.8-5.5)	3.7 (3.1-4.4)	5.0 (4.5-5.7)
Full cream milk (daily)	3238	Crude	19.1 (15.2-24.0)	11.2 (8.7-14.3)	7.2 (5.5-9.4)	6.1 (4.5-8.3)	6.3 (4.5-8.9)	6.2 (4.2-9.2)	13.2 (10.9-16.0)
		Adjusted†	19.7 (15.6-24.8)	11.3 (8.8-14.6)	7.2 (5.5-9.4)	6.1 (4.5-8.3)	6.3 (4.4-8.9)	6.1 (4.1-9.1)	13.3 (11.0-16.2)
Semi-skimmed milk (daily)	3238	Crude	9.5 (8.1-11.2)	4.6 (4.0-5.4)	3.4 (2.9-3.9)	3.2 (2.7-3.7)	2.6 (2.3-3.0)	2.5 (2.1-2.8)	4.1 (3.7-4.6)
		Adjusted†	9.8 (8.3-11.6)	4.7 (4.0-5.5)	3.4 (3.0-4.0)	3.2 (2.8-3.7)	2.6 (2.3-3.0)	2.5 (2.1-2.9)	4.1 (3.7-4.6)
Butter (daily)	3242	Crude	90.6 (62.1-132.3)	58.4 (40.4-84.6)	44.5 (31.1-63.6)	45.8 (31.4-66.9)	39.3 (26.9-57.7)	34.6 (23.0-52.1)	53.8 (40.8-71.0)
		Adjusted†	89.6 (61.1-131.5)	56.7 (38.7-83.0)	43.2 (29.7-62.8)	44.3 (30.2-65.1)	38.1 (25.8-56.2)	33.2 (21.8-50.5)	52.2 (39.3-69.5)
Margarine (daily)	3232	Crude	9.9 (8.1-12.0)	7.2 (6.0-8.7)	5.4 (4.5-6.4)	3.7 (3.1-4.4)	3.2 (2.7-3.7)	2.8 (2.4-3.3)	4.6 (4.1-5.2)
		Adjusted†	9.9 (8.1-12.0)	7.2 (6.0-8.7)	5.3 (4.4-6.4)	3.6 (3.1-4.3)	3.1 (2.7-3.7)	2.8 (2.4-3.3)	4.6 (4.0-5.2)

*Number of children included in GEE analyses.

†Analyses adjusted for potential confounders sex, maternal education, parental atopy, allergic sibling, maternal smoking during pregnancy, smoking in the home at baseline, breastfeeding, birth weight, maternal overweight and region.

Strongest overall tracking was observed for high butter consumption (table 5.4). At each time point this habit was practiced by approximately 5% of the population (table 5.2), which were often the same children. Long-term daily butter consumption was observed for almost 2% of the children. Lowest tracking was observed for high fish, semi-skimmed milk and margarine consumption. However, the proportion of children reporting high consumption of semi-skimmed milk did not strongly change over time, while the proportion of high fish and margarine consumption moderately increased. Long-term high consumption of semi-skimmed milk, margarine and fish was observed for ca. 25%, 43% and 7% of the children respectively (table 5.3). The strongest decrease was observed for full cream milk consumption. Approximately one third of the children daily consumed full cream milk when they were two years old, but less than 5% of the children did this when they were eight years old. Only 2% of the children daily consumed full cream milk during the entire time period. Yet, the overall tracking for high full cream milk consumption was relatively strong, meaning that children, who still daily consumed full cream milk when they were 8 years old, were most likely the ones who already did so at age 2. High fruit and vegetable consumption also decreased considerably over time (from 68% to 58% and from 57% to 37% respectively), tracking was relatively moderate. Thirty percent of the children consistently reported high consumption of fruit, while only 13% consistently reported high consumption of cooked vegetables during the entire time period.

Adjustment for potential confounders did not strongly alter the tracking of high consumption of the different food groups. However, we have assessed interaction by maternal educational level and found that the overall tracking for high fish consumption was significantly lower in children of mothers with a lower educational level (OR 4.0; 95% CI 3.0-5.0) compared to children of mothers with an intermediate or high educational level (OR 5.5; 95% CI 4.8-6.3). For high fruit and margarine consumption, the overall tracking was significantly higher in children of mothers with a higher educational level compared to children of mothers with an intermediate or low educational level (7.0 (5.6-8.7) vs 5.5 (4.8-6.4) and 6.2 (5.0-7.6) vs 3.9 (3.3-4.5)) respectively.

Figure 5.1 and figure 5.2 show associations between high consumption at different initial ages (age 2, 3, 4, 5, 6 and 7) and high consumption at further follow-up. For fruit, vegetables, brown/wholemeal bread and fish, associations between high consumption at equally spaced age points are similar. For instance, for high fruit consumption the association between age 2 and age 3 (OR 10.0; 95% CI 8.4-11.9) is similar to the association between age 4 and 5 (OR 10.6; 95% CI 8.9-11.9), and the association between age 5 and 7 (11.7 (9.8-13.8)) is similar to the association between 6 and 8 (11.4 (9.5-13.7)). For full cream milk, semi-skimmed milk, butter and margarine associations between high consumption at equally spaced time points become stronger when the child grows older. For instance, for high semi-skimmed milk consumption the association between age 2 and 3 (OR 9.8; 95% CI 8.3-11.6) is weaker than the association between age 4 and 5 (OR 19.8; 95% CI 16.4-23.9) and the association between age 3 and 5 (7.6 (6.4-8.9)) is weaker than the association between age 5 and 7 (15.6 (13.0-18.7)). This means that for some foods (fruit, vegetables, brown/wholemeal bread and fish) consumption habits do not become less

variable over time, while for full cream milk, semi-skimmed milk, butter and margarine, the consumption habits of, for instance, five-year-olds are more representative for consumption habits at later follow-up than consumption habits of two-year-olds. Furthermore, we can see in this figure that the association of for instance, high vegetable and fish consumption between age 7 and 8 years is similar to the association between age 2 and 8 years, whereas for high semi-skimmed milk and margarine consumption the association between age 7 and 8 is much stronger than the association between age 2 and 8 years.

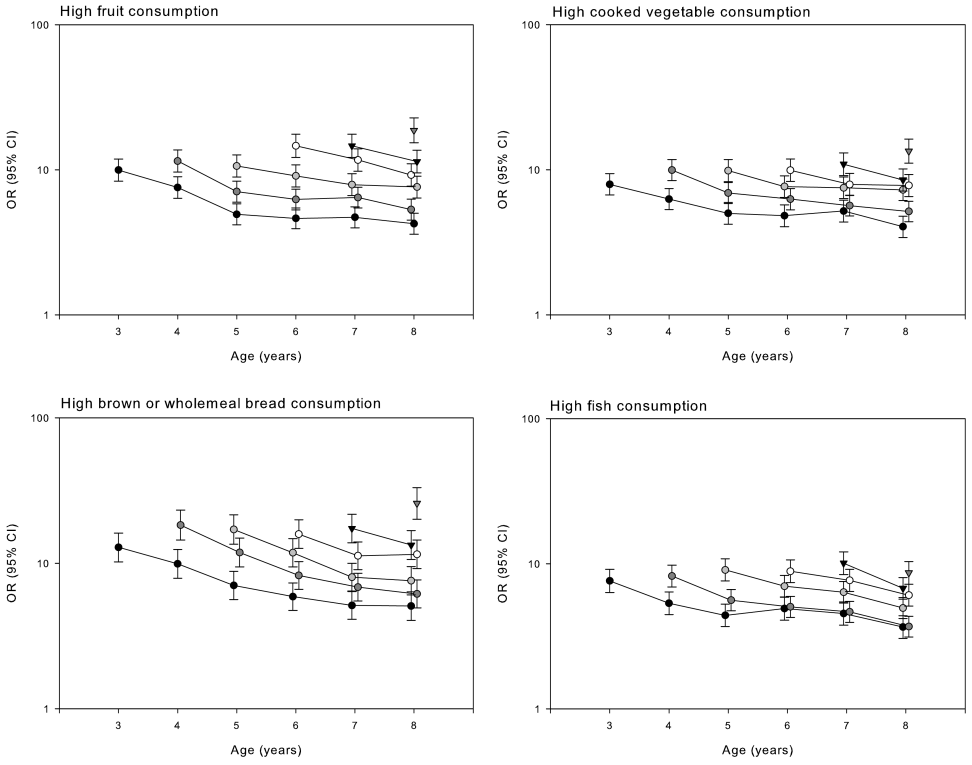

Figure 5.1: *Associations of high fruit, cooked vegetables, brown/wholemeal bread, and fish consumption between ages 2 (•), 3 (•) , 4 (•), 5 (○), 6 (▼) and 7 (▼) and subsequent follow-up.*

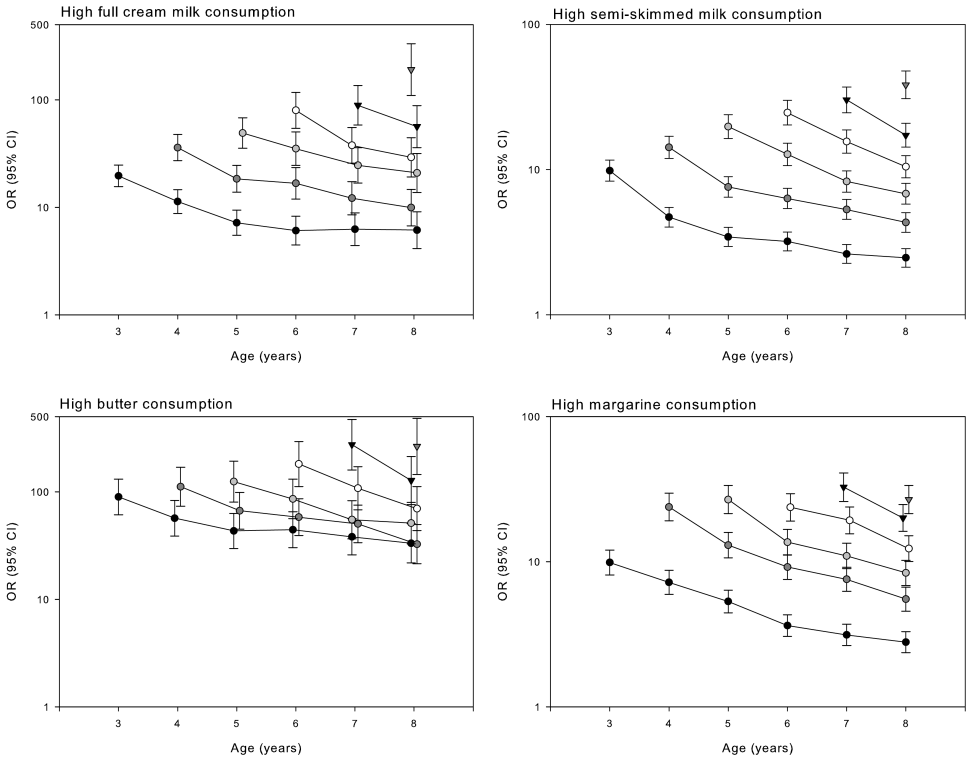

Figure 5.2: *Associations of high full cream milk, semi-skimmed milk, butter and margarine consumption between ages 2 (•), 3 (•) , 4 (•), 5 (∘), 6 (▼) and 7 (▼) and subsequent follow-up. (Note that y-axis scale for full cream milk and butter are different from y-axis scales of other foods)*

5.4 Discussion

In the present study we have investigated the stability of dietary habits from 2 to 8 years of age in children participating in the PIAMA birth cohort. The prevalence of high consumption decreased for full cream milk, fruit and vegetables, while it increased for fish and margarine. Highest tracking was seen for butter consumption. Tracking of fruit, cooked vegetables, fish, margarine and semi-skimmed milk consumption was moderate to low. For tracking of fruit, fish and margarine consumption, significant interaction by maternal educational level was found.

Tracking of dietary habits can be assessed in different ways. Some studies assess tracking by calculating the stability in rank at the group level, and/or the proportion of participants that stay in the same group in the final measurement,[4–6] while other studies work with (weighted) kappa statistics (κ)[7–9] or correlation coefficients.[10] The

disadvantage of these techniques is that only information of two measurements can be used, that the influence of potential confounding factors cannot be taken into account, and that the interpretation of these coefficients is often difficult.[11] The advantage of using longitudinal GEE analyses to estimate tracking is that all available longitudinal data can be used, and that the estimates can be corrected for potential confounding factors. The GEE method also corrects for within-subject correlations. Yet, we need to take into account that the magnitude of the tracking odds ratios depends on the initial age of the participants, the number of repeated measurements, the time period(s) between the measurements and the length of the total time period under consideration, when comparing results from different studies.[12]

One of the problems in longitudinal studies can be incomplete follow-up and non-response. Consumption frequencies in table 5.2 and table 5.3 are based on participants with complete dietary information over time. Non-responding mothers were more likely to have a lower educational level, smoked during pregnancy or smoked in the home, and to be from region south-west, while they were less likely to have breast fed their child. The observed decrease in high consumption of fruit and vegetables over time might thus be an underestimation of the decrease in the total study population. Our results showed significant interaction of maternal educational level in tracking of high fruit, fish and margarine consumption. Socioeconomic factors like educational level may thus be important determinants of the stability of consumption habits of certain foods.

Another issue was the possibility of measurement error, which plays an important role in the evaluation of tracking. Lowest tracking coefficients are found for variables with the highest measurement error, i.e. the lowest reproducibility.[11] Data on food consumption were measured by food frequency questionnaires consisting of approximately 30 to 35 different foods or food groups. Reproducibility of food frequency questionnaires depends on the specification of the items and the time-lag between the repeated measurements. Differences between repeated measurements increase when questions are more specific, for instance when enquiring the consumption of more specific foods (e.g. apples, oranges, bananas) instead of larger food groups (e.g. fruit), and when the time-lag increases.[13] Food groups investigated in this study are not very specific, mostly the consumption of larger food groups is asked for, so we expect reasonable reproducibility. The time-lags between the repeated measurements were equally spaced and relatively small - 1 year.

A limitation of FFQ-derived estimates is that they are susceptible to dietary misreporting. Participants may have difficulties in recalling their dietary habits, the questions may be too difficult, or they may give socially desirable answers, which leads to misclassification of intake.[14] However, we assume that this possible misreporting is not time-dependent so it probably does not lead to lower tracking coefficients. To date, few longitudinal studies on tracking of dietary habits in children are available. It is difficult to compare our results to those of other studies, because most of them use different methodology and/or investigate tracking of behavior from childhood or adolescence into adulthood. The decrease and low to moderate tracking of fruit and vegetable consumption when children grow older has also been observed by other studies.[6,9,15] A longitudinal study investigating the tracking of fruit and vegetable

consumption from adolescence into adulthood showed that fruit and vegetable intakes according to recommendations at younger age increase the likelihood of eating according to recommendations later on in life two- to sixfold,[16] tracking odds ratios of similar magnitude as found in the present study.

The assumption that dietary consumption estimated at a certain time point reflects the habitual intake during childhood is not valid for all foods. Childhood diet changes over time, so longitudinal studies in children can provide better estimates of dietary exposure, and are indispensable to assess the effect of changes in diet on children's health.

5.5 Appendix

The equation used for predictability analysis is

$$\ln\left[\frac{P(Y_{it}=1)}{1-P(Y_{it}=1)}\right] = \beta_0 + \beta_1 Y_{it1} + \beta_2 t + \sum_{j=1}^{J} \beta_{3j} X_{ijt} + \sum_{k=1}^{K} \beta_{4k} Z_{ik}, \qquad (5.1)$$

where $P(Y_{it} = 1)$ is the probability that the observations at t_2 to t_m of subject i equals 1 (where m is the number of measurements and 1 means that subject i belongs to the group of interest), Y_{it_1} is the (first) initial observation of subject i at t_1, β_0 is the intercept, β_1 is the regression coefficient used as predictability coefficient, t is time, β_2 is the regression coefficient for time, X_{ijt} is the time-dependent covariate j for subject i, β_{3j} is the regression coefficient for time-dependent covariate j, J is the number of time-dependent covariates, Z_{ik} is the time-independent covariate k for subject j, β_{4k} is the regression coefficient for time-dependent covariate k, and K is the number of time-independent covariates.[3]

5.6 References

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Chapter 6

Childhood nutrition and asthma, atopy and bronchial hyperresponsiveness at 8 years of age: the PIAMA birth cohort study

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Abstract

Background Most studies relating diet with childhood asthma or atopy assess dietary exposure at one point in time to health outcomes at the same point in time or a (few) year(s) later. The use of longitudinal dietary data could give insight in effects of long-term dietary exposure, and of differences in effects of dietary habits at early and at later age.

Objectives The objectives of this study were to investigate if symptoms of asthma or atopy at 8 years of age were associated with long-term dietary exposure from 2 to 8 years of age, and whether associations differed for consumption at early or later age.

Methods The PIAMA birth cohort consists of a baseline population of 4146 participants followed for 8 years. Longitudinal dietary data were derived from annual questionnaires. Foods of interest were fruit, vegetables, brown/wholemeal bread, fish, milk, butter and margarine. Multivariate logistic regression analyses were used to investigate the associations between long-term consumption, and early (at 2 and 3 years of age) and late (at 7 and 8 years of age) consumption on symptoms of asthma, atopy and BHR at 8 years of age.

Main results Long-term high fruit consumption was borderline inversely associated with inhaled steroid use (OR 0.67; 95% CI 0.43-1.03), asthma symptoms (OR 0.74; 95% CI 0.54-1.00), and sensitisation to inhalant allergens (OR 0.79; 95% CI 0.58-1.06). Furthermore we observed a beneficial association between high fruit consumption and asthma symptoms at later age, but not at early age. No associations were found between early, late or long-term dietary consumption of the other investigated foods on symptoms of asthma or atopy at 8 years of age.

Conclusion Daily fruit consumption at later age was associated with a decreased risk of wheeze and asthma symptoms at 8 years of age. The timing of food consumption had no effect on the development of asthma and allergy.

6.1 Introduction

"An apple a day keeps the doctor away!" This and many other adages refer to people's awareness of the relation between nutrition and health. Epidemiologists have studied this relation for quite some time. First, to identify essential nutrients, later to discover effects of the change in diet due to Western civilization. This prosperity associated change in diet, and more specifically the reduced consumption of antioxidant rich foods and change in dietary fat intake, has been linked to the major increase in asthma and allergic disease in the last decades.[1,2]

Previous studies have reported beneficial associations between a higher consumption of fruit,[3–5] vegetables,[4] fish,[5,6] full fat dairy products,[7] and wholegrain products,[7,8] and symptoms of asthma or allergy in children, while harmful associations have been reported for margarine and salt intake.[9,10] It has been hypothesised that dietary exposure in early life (from conception to 2 years) might be particularly important in the development of childhood asthma, because the airways and immune system are developing during this period.[11] Most of the previous studies that assessed the effect of diet on asthma in children, relate dietary data that is obtained at one point in time to health outcomes at the same point in time or a (few) year(s) later. Results of these studies do not give information about the effects of long-term dietary exposure or differences in effects of dietary habits at early or later age. The use of longitudinal dietary data would give more insight in these effects, which may be crucial for (the timing of) dietary preventive strategies for childhood asthma and allergy.

The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study has annual follow-up dietary data from birth to 8 years of age. The aim of the present study was to investigate the effects of long-term dietary exposure from 2 to 8 years of age, and dietary habits at early and later age on symptoms and clinical outcomes of asthma and atopy at 8 years of age.

6.2 Methods

6.2.1 Study population and design

In 1996, a large birth cohort study was set up to investigate the Prevention and Incidence of Asthma and Mite Allergy (PIAMA). This birth cohort study started off with the screening of 10,232 pregnant women in prenatal health clinics. Resulting from this screening, 2949 women were defined as atopic, while 7283 were defined as non-atopic.[12] 2779 atopic women, and 5083 non-atopic women were invited to participate in the study. 1327 atopic women and 2819 non-atopic women agreed to participate. In the intervention part of PIAMA, which was designed to study the effect of mite-allergen avoidance by means of mite-impermeable mattress- and pillow covers, only children born to atopic mothers ('high-risk' children) were enrolled. In the observational natural history part, children of atopic as well as children of non-atopic mothers ('low-risk' children) were enrolled. Due to oversampling of non-atopic mothers in the natural history part the proportion of atopic mothers in the baseline

study population was similar to the proportion of atopic mothers in the screened population (∼30%).

The study started with 3,963 newborn children, because 183 mothers (∼4.5%) were lost to follow up before any data on the child had been collected. Questionnaires containing the ISAAC core questions on asthma, rhinitis and eczema[13] as well as various questions on lifestyle factors (nutrition, pets, home characteristics etc.) were sent to the participants at 3 months of age, and yearly from 1 to 8 years of age. Further details of the design of the PIAMA study have been published previously.[14]

6.2.2 Childhood nutrition

Longitudinal data on the child's diet were derived from yearly questionnaires from 2 to 8 years of age. These questionnaires enquired about the frequency of consumption of approximately 30 to 35 different foods or food groups in the preceding month. The foods and food groups of interest in the present study were the ones high in antioxidants (fruit, vegetables and brown/wholemeal bread), n-3 fatty acids (fish), n-6 fatty acids (margarine) and milk fat (full cream milk, semi-skimmed milk and butter). Answer options for the frequency of consumption were: (1) never, (2) less than once a week, (3) one to two days a week, (4) three to five days a week, and (5) six to seven days a week. These answer options were grouped into three categories: rare consumption (option 1 and 2), regular consumption (option 3 and 4) and daily consumption (option 5).

In the analyses regarding the effects of dietary habits on health outcomes, dichotomous variables for dietary exposure were used (high vs low consumption). High consumption was defined as daily consumption, while low consumption was defined as regular plus rare consumption. This was done for all investigated foods and food groups except for fish, because the proportion of daily fish consumers was very small. For fish, high consumption was defined as daily + regular consumption (once a week or more), while low consumption was defined as rare consumption.

High consumption at early age was defined as high consumption at both 2 and 3 years of age, and compared with children whose parents did not consistently report high consumption at both 2 and 3 years of age. High consumption at later age was defined as high consumption at both 7 and 8 years of age, and compared with children whose parents did not consistently report high consumption at both 7 and 8 years of age. The dietary exposure variable 'long-term consumption' was defined for consumption during the entire period from 2 to 8 years of age. This variable was constructed by calculating the average of the questionnaire answer options (1 to 5) given at each age point. For all investigated foods except fish, an average of 5 (daily consumption at each age point) was defined as 'high' long-term consumption. An average of at least 4.5 but smaller than 5 was defined as 'intermediate' long-term consumption, whereas an average smaller than 4.5 was defined as 'low' long-term consumption. For fish, an average of 3 or higher was defined as 'high' long-term consumption, an average lower than 3 but higher than or equal to 2.5 was defined as 'intermediate' long-term consumption, whereas an average lower than 2.5 was defined as 'low' long-term consumption.

The group of children with 'high' long-term consumption of full cream milk and butter were very small so for the analyses of long-term consumption of these foods the 'high' and 'intermediate' categories were combined.

6.2.3 Health outcomes

The child's health outcomes of interest were wheeze, dyspnea, inhaled steroid use and the composite variable asthma symptoms derived from the 8-year questionnaire, and sensitisation against inhalant and food allergens, and bronchial hyperresponsiveness (BHR) assessed by medical examination at 8 years of age. The composite variable 'asthma symptoms' was based on questions about wheeze, dyspnea and inhaled steroid use in the last 12 months. A child was defined as having 'asthma symptoms' when the parents reported one or more attacks of wheeze, and/or one or more events of dyspnea and/or prescription of inhaled steroids for respiratory problems in the last 12 months. A child who had none of these characteristics was defined as not having 'asthma symptoms'. Children who were still participating in the study were invited for either an extensive or small medical examination. Children from the intervention part, the 'high-risk' natural history part, and a random sample of the 'low-risk' natural history part, drawn at the beginning of the PIAMA study, were invited for the extensive medical examination conducted in hospital. Participants who were not able to come to the hospital clinic and the other group of 'low-risk' natural history children were invited for a smaller medical examination either at a local community health centre or at home. Both the extensive and small medical examination at 8 years of age included blood sampling for the assessment of total and specific IgE levels. Children were considered to be sensitised against inhalant allergens if one or more allergen specific IgE levels to house dust mite (*Dermatophagoides pteronyssinus*), cat, dog, birch (*Betula verrucosa*), grass (*Dactylis glomerata*) and fungus (*Alternaria alternata*) were equal to or higher than 0.35 IU/ml. Sensitisation to food allergens was defined as a high level of allergen specific IgE to milk or egg (also $\geq$0.35 IU/ml). A metacholine provocation test as indicator for bronchial hyperresponsiveness (BHR) was only conducted during the extensive medical examination in hospital. Children were defined as having BHR when the cumulative dose of metacholine bromide causing a 20% decrease in FEV_1 (PD20) was $\leq$0.61 mg.

6.2.4 Statistical analyses

Univariate and multivariate logistic regression analyses were used to assess associations between the different dietary habits during childhood and health outcomes at 8 years of age. 'Intermediate' and 'high' long-term consumption were included as dummy variables in the models, with 'low' long-term consumption serving as reference category. Children with 'high' consumption at early age or later age were compared to children with'low' consumption at early age or later age, respectively. Covariates included as potential confounding factors in the multivariate model were: sex, parental atopy, allergic sibling, maternal education (low, intermediate, high), maternal smoking during pregnancy, smoking in the home by mother, father or oth-

ers at age 8 years, breast feeding (yes/no), presence of older siblings, birth weight ($\leq$3000 g, 3001 to 3999 g, $\geq$4000 g), overweight mother (maternal BMI $\geq$25 kg/m^2 assessed at 1 year follow-up), region (north, central, south-west) and study arm (intervention study, 'high-risk' or 'low-risk' natural history study). Associations with p-values smaller than 0.05 were regarded as statistically significant, whereas p-values smaller than 0.10 were regarded as borderline statistically significant. All analyses were carried out using SAS for Windows version 9.1 (SAS Institute, Cary, NC, USA).

6.3 Results

At 8 years follow-up, questionnaire information was obtained for 3320 children. 1682 participants were invited for the extensive medical examination while 1840 were invited for the smaller medical examination at home, 1264 and 1014 participants agreed for the extensive and small examination respectively. During the extensive medical examination, 1013 children performed a metacholine provocation test, of whom 960 were successful. IgE levels in blood samples were analysed for 1714 children. Complete dietary data from 2 to 8 years of age on at least one of the investigated food groups were obtained for 2870 children. The characteristics of respondents at baseline compared to complete cases with questionnaire information at 8 years of age and complete cases with IgE data at 8 years of age are shown in table 6.1.

Children with complete data at 8 years of age were less likely to have a mother with atopy and a low educational level, a mother who smoked during pregnancy, or to be from region south-west compared to children at baseline. Furthermore, these children were more likely to have a mother with a high educational level, and had a higher mean birth weight compared to children at baseline. Children with IgE data at 8 years of age were less likely to be from region north. Yet, the magnitude of the differences between populations was small.

Table 6.2 shows the consumption frequency categories 'daily', 'regularly' and 'rarely' for fresh fruit, cooked vegetables, brown or wholemeal bread, fish, full cream milk, semi-skimmed milk, butter, and margarine consumption from 2 to 8 years of age. The proportion of children who daily consumed fruit and vegetables decreased over time. The strongest decrease was observed for daily consumption of full cream milk, from 31.9% of the children at 2 years of age, to 4.4% at 8 years of age. The proportion of children who daily consumed semi-skimmed milk, brown/wholemeal bread or butter remained stable over time, while the proportion of children who at least regularly consumed fish increased slightly.

Table 6.1: *Characteristics of the study population at baseline and complete cases at 8 years of age*

Characteristics	**Respondents at baseline**	**Complete cases at 8 yrs**	**Complete cases with IgE data at 8 yrs**
	N=3963	**N=2609**	**N=1345**
	n (%)	**n (%)**	**n (%)**
Female child	1911 (48.2)	1280 (49.1)	662 (49.2)
Atopic mother	821 (20.7)	469 (18.0*)	314 (23.4*)
Atopic father	801 (20.2)	553 (21.2)	265 (19.7)
Both parents atopic	416 (10.5)	233 (8.9*)	158 (11.8*)
Allergic sibling	756 (19.2)	491 (18.8)	274 (20.4)
Maternal education low	894 (23.5)	525 (20.1*)	262 (19.5)
Maternal education intermediate	1582 (41.5)	1106 (42.4)	560 (41.6)
Maternal education high	1331 (35.0)	978 (37.5*)	523 (38.9)
Maternal smoking during pregnancy	696 (17.8)	396 (15.2*)	194 (14.4)
Smoking in the home at 8 years	-	364 (14.0)	175 (13.0)
Ever breastfed	3200 (82.1)	2180 (83.6*)	1139 (84.7)
Presence of older siblings	1994 (50.7)	1315 (50.4)	700 (52.0)
Birth weight low ($\leq$3000 g)	353 (13.8)	320 (12.3*)	156 (11.6)
Birth weight normal (3001-3999 g)	2677 (68.2)	1795 (68.8)	931 (69.2)
Birth weight high ($\geq$4000 g)	702 (18.0)	494 (18.9)	258 (19.2)
Maternal overweight (BMI $\geq$25 kg/m^2)	886 (25.1)	650 (24.9)	328 (24.4)
Region north	1231 (31.1)	827 (31.7)	240 (25.3*)
Region central	1586 (40.0)	1109 (42.5)	744 (47.9)
Region south-west	1146 (28.9)	673 (25.8*)	361 (26.8)
Intervention study	781 (19.7)	384 (14.7)	281 (20.9*)
'High-risk' Natural history study	456 (11.5)	318 (12.2)	191 (14.2*)
'Low-risk' Natural History study	2726 (68.8)	1907 (73.1)	873 (64.9)

*$p<0.05$: complete cases included in the analyses vs non-complete or complete cases with IgE data vs complete cases without IgE data

Table 6.2: *Consumption frequencies for selected foods and food groups from 2 to 8 years of age*

Food	N*	Frequency	Age 2 n (%)	Age 3 n (%)	Age 4 n (%)	Age 5 n (%)	Age 6 n (%)	Age 7 n (%)	Age 8 n (%)
Fresh fruit	2815	rarely	103 (3.6)	91 (3.2)	74 (2.6)	92 (3.4)	91 (3.2)	86 (3.1)	99 (3.5)
		regularly	795 (28.4)	959 (34.1)	974 (34.6)	1021 (36.3)	1030 (36.6)	1118 (39.7)	1081 (38.4)
		daily	1917 (68.1)	1765 (62.7)	1767 (62.8)	1702 (60.5)	1694 (60.2)	1611 (57.2)	1635 (58.1)
Cooked vegetables	2793	rarely	74 (2.6)	130 (4.6)	106 (3.8)	82 (2.9)	72 (2.6)	57 (2.0)	57 (2.0)
		regularly	1130 (40.5)	1387 (49.7)	1495 (53.5)	1692 (60.6)	1733 (62.0)	1713 (61.3)	1695 (60.7)
		daily	1589 (56.9)	1276 (45.7)	1192 (42.7)	1019 (36.5)	988 (35.4)	1023 (36.7)	1041 (37.3)
Brown/wholemeal bread	2839	rarely	87 (3.1)	91 (3.2)	95 (3.3)	118 (4.1)	113 (4.0)	106 (3.7)	117 (4.1)
		regularly	329 (11.6)	348 (12.3)	368 (13.0)	391 (13.8)	445 (15.7)	432 (15.2)	429 (15.1)
		daily	2423 (85.3)	2400 (84.5)	2376 (83.7)	2330 (82.1)	2281 (80.3)	2301 (81.1)	2293 (80.8)
Fish	2716	rarely	2094 (77.1)	1881 (69.3)	1789 (65.9)	1838 (67.7)	1728 (63.6)	1702 (62.7)	1685 (62.0)
		regularly	617 (22.7)	821 (30.2)	920 (33.9)	874 (32.2)	984 (36.2)	1009 (37.2)	1025 (37.7)
		daily	5 (0.2)	14 (0.5)	7 (0.3)	4 (0.2)	4 (0.2)	5 (0.2)	6 (0.2)
Full cream milk	2756	rarely	1720 (62.4)	2037 (73.9)	2203 (79.9)	2319 (84.1)	2431 (88.2)	2496 (90.6)	2550 (92.5)
		regularly	157 (5.7)	174 (6.3)	187 (6.8)	157 (5.7)	135 (4.9)	102 (3.7)	84 (3.1)
		daily	879 (31.9)	545 (19.8)	366 (13.3)	280 (10.2)	190 (6.9)	158 (5.7)	122 (4.4)
Semi-skimmed milk	2778	rarely	1014 (36.5)	860 (31.0)	744 (26.8)	729 (26.2)	752 (27.1)	770 (27.7)	766 (27.6)
		regularly	305 (11.0)	357 (12.8)	400 (14.4)	469 (16.9)	493 (17.7)	439 (15.8)	467 (16.8)
		daily	1459 (52.5)	1561 (56.2)	1634 (58.8)	1580 (56.9)	1533 (55.2)	1569 (56.5)	1545 (55.6)
Butter	2818	rarely	2471 (87.7)	2461 (87.3)	2457 (87.2)	2466 (88.2)	2489 (88.3)	2482 (88.1)	2501 (88.7)
		regularly	160 (5.7)	175 (6.2)	196 (6.9)	163 (5.8)	189 (6.7)	201 (7.1)	203 (7.2)
		daily	187 (6.6)	182 (6.5)	165 (5.9)	169 (6.0)	140 (5.0)	135 (4.8)	114 (4.1)
Margarine	2794	rarely	551 (19.7)	270 (9.7)	264 (9.5)	304 (10.9)	335 (12.0)	362 (13.0)	388 (13.9)
		regularly	418 (15.0)	321 (11.5)	320 (11.5)	342 (12.2)	341 (12.2)	372 (13.3)	417 (14.9)
		daily	1825 (65.3)	2203 (78.8)	2210 (79.1)	2148 (76.9)	2118 (75.8)	2060 (73.7)	1989 (71.2)

*Number of children with dietary information on that particular food from 2 to 8 years of age.

Table 6.3 shows the prevalence of 'low', 'intermediate' and 'high' long-term consumption over the period from 2 to 8 years of age. For fruit, vegetables and brown/wholemeal bread, the proportion of children with 'intermediate' or 'high' long-term consumption was higher than the proportion of children with 'low' long-term consumption. The proportion of 'high' long-term consumption was largest for brown/wholemeal bread and margarine, whereas the proportion of children with 'low' long-term consumption was largest for butter, full-cream milk, fish, cooked vegetables and semi-skimmed milk.

Table 6.3: *Prevalence of long-term consumption patterns of different foods over the period from 2 to 8 years of age*

Food consumption	**N***	**Low long-term n (%)**	**Intermediate long-term n (%)**	**High long-term n (%)**
Fresh fruit	2815	1119 (39.8)	851 (30.2)	845 (30.0)
Cooked vegetables	2793	1740 (62.3)	696 (24.9)	357 (12.8)
Brown/wholemeal bread	2839	551 (19.4)	591 (20.8)	1697 (59.8)
Fish	2716	1920 (70.7)	613 (22.6)	183 (6.7)
Full cream milk	2756	2665 (96.7)	32 (1.2)	59 (2.1)
Semi-skimmed milk	2778	1714 (61.7)	376 (13.5)	688 (24.8)
Butter	2818	2732 (96.9)	41 (1.5)	45 (1.6)
Margarine	2794	1057 (37.3)	545 (19.5)	1192 (42.7)

*Number of children with dietary information on that particular food at all ages.

Table 6.4 shows the prevalence of high consumption at early and later age. For all investigated food groups except fish, semi-skimmed milk and margarine, the prevalence of high consumption at early age was higher than at later age.

Table 6.4: *Prevalence of high consumption at early and later age*

Food consumption	**N***	**High consumption at early age n (%)**	**High consumption at later age n (%)**
Fresh fruit	2990	1616 (54.1)	1444 (48.3)
Cooked vegetables	2996	1118 (37.3)	805 (26.9)
Brown/wholemeal bread	3016	2340 (77.6)	2246 (74.5)
Fish	2956	458 (15.5)	751 (25.4)
Full cream milk	2968	488 (16.4)	103 (3.5)
Semi-skimmed milk	2981	1240 (41.6)	1446 (48.5)
Butter	2990	135 (4.5)	99 (3.3)
Margarine	2970	1796 (60.5)	1933 (65.1)

*Number of children with dietary information at age 2, 3, 7 and 8 years.

Prevalences of symptoms of asthma, atopy and BHR at 8 years of age are shown in table 6.5. The most prevalent symptom at 8 years among children with questionnaire data was dyspnea (9.1%). Of the children with IgE data, ∼30% was sensitised to

inhalant allergens while ∼15% was sensitised to milk or egg. The proportion of children with a positive metacholine provocation test was almost 40%. However, the proportion of 'high risk' children in the subgroup that performed metacholine provocation tests was higher than in the total study population (∼65%).

Table 6.5: *Prevalence of health outcome variables at 8 years of age*

Health outcome	**N***	**n (%)**
Wheeze in the last 12 months	3320	221 (6.6)
Dyspnea in the last 12 months	3320	303 (9.1)
Inhalation steroids in the last 12 months	3320	211 (6.4)
Asthma symptoms†	3320	437 (13.2)
Sensitisation to inhaled allergens‡	1714	550 (32.1)
Sensitisation to food allergens§	1714	285 (16.6)
Bronchial hyperresponsiveness	1013	402 (39.7)

*Number of children with questionnaire information, IgE data or metacholine provocation test respectively.

†Composite variable of wheeze, dyspnea or steroid use.

‡House dust mite, cat dog, grass, birch or fungus.

§Milk or egg.

Adjusted associations between long-term consumption and wheeze, dyspnea, steroid use and asthma symptoms are shown in table 6.6. Adjusted associations between long-term consumption and sensitisation to inhalant allergens, food allergens and BHR are shown in table 6.7. There were no significant associations between 'intermediate' or 'high' long-term consumption of cooked vegetables, brown/wholemeal bread, fish, full cream milk, semi-skimmed milk, butter and margarine compared to 'low' long-term consumption, on symptoms of asthma, atopy or BHR at 8 years of age. 'High' long-term fresh fruit consumption was inversely associated with inhaled steroid use and the composite variable 'asthma symptoms' (borderline significant), whereas 'intermediate' long-term fruit consumption was inversely associated with sensitisation to inhalant allergens. Inclusion of potential confounding factors in the different models did not strongly alter the results.

Table 6.6: *Adjusted associations of intermediate and high long-term consumption versus low long-term consumption and health outcomes at 8 years of age*

Food	Long-term consumption	Wheeze	Dyspnea	Steroid use	Asthma symptoms*
		OR (95% CI)†	OR (95% CI)†	OR (95% CI)†	OR (95% CI)†
Fresh fruit	Intermediate	0.77 (0.52-1.14)	0.91 (0.65-1.28)	0.89 (0.60-1.31)	0.85 (0.64-1.13)
	High	0.81 (0.54-1.22)	0.85 (0.60-1.22)	0.67 (0.43-1.03)	0.74 (0.54-1.00)
Cooked vegetables	Intermediate	1.09 (0.73-1.62)	0.95 (0.68-1.34)	0.89 (0.59-1.34)	1.00 (0.75-1.33)
	High	1.50 (0.95-2.36)	1.18 (0.78-1.79)	0.92 (0.54-1.56)	1.20 (0.84-1.71)
Brown/wholemeal bread	Intermediate	1.02 (0.62-1.67)	0.92 (0.60-1.42)	0.96 (0.57-1.61)	0.90 (0.62-1.30)
	High	0.79 (0.51-1.23)	0.80 (0.55-1.16)	0.86 (0.55-1.35)	0.75 (0.55-1.03)
Fish	Intermediate	1.07 (0.72-1.59)	1.12 (0.79-1.58)	1.55 (1.05-2.28)‡	1.27 (0.95-1.69)
	High	1.44 (0.79-2.63)	1.37 (0.80-2.32)	0.85 (0.38-1.88)	1.27 (0.79-2.05)
Full cream milk	Intermediate + High	2.01 (0.93-4.32)	1.18 (0.53-2.64)	0.94 (0.34-2.65)	1.31 (0.68-2.52)
Semi-skimmed milk	Intermediate	1.38 (0.89-2.15)	0.97 (0.64-1.47)	1.11 (0.68-1.79)	1.02 (0.72-1.45)
	High	0.79 (0.51-1.21)	0.89 (0.63-1.26)	0.92 (0.61-1.40)	0.92 (0.69-1.24)
Butter	Intermediate + High	0.85 (0.30-2.40)	0.76 (0.30-1.92)	0.97 (0.35-2.74)	0.73 (0.33-1.63)
Margarine	Intermediate	0.64 (0.39-1.05)	0.96 (0.65-1.42)	0.91 (0.56-1.46)	0.89 (0.63-1.24)
	High	0.91 (0.64-1.30)	0.89 (0.65-1.22)	0.97 (0.67-1.40)	0.90 (0.69-1.18)

*Composite variable of wheeze, dyspnea or inhaled steroid use.

†Multivariate models are adjusted for sex, maternal educational level, parental atopy, maternal smoking during pregnancy, smoking in the house at age 8 years, breast feeding, presence of older siblings, birth weight, overweight mother, allergic sibling, region and study arm.

‡$p<0.05$

Table 6.7: *Adjusted associations of intermediate and high long-term consumption versus low long-term consumption and health outcomes at 8 years of age*

Food	Long-term consumption	Sensitisation to inhalant allergens* OR (95% CI)‡	Sensitisation to food allergens† OR (95% CI)‡	Bronchial Hyperresponsiveness OR (95% CI)‡
Fresh fruit	Intermediate	0.68 (0.51-0.92)§	0.74 (0.50-1.08)	0.94 (0.65-1.37)
	High	0.79 (0.58-1.06)	0.78 (0.53-1.15)	0.95 (0.64-1.41)
Cooked vegetables	Intermediate	0.91 (0.68-1.21)	0.93 (0.64-1.33)	0.88 (0.61-1.28)
	High	0.98 (0.68-1.42)	0.98 (0.62-1.55)	0.97 (0.61-1.55)
Brown/wholemeal bread	Intermediate	1.16 (0.78-1.72)	1.33 (0.77-2.30)	0.60 (0.36-0.99)§
	High	1.07 (0.75-1.51)	1.43 (0.89-2.32)	0.71 (0.46-1.10)
Fish	Intermediate	0.91 (0.68-1.23)	0.95 (0.65-1.39)	0.83 (0.57-1.20)
	High	0.92 (0.58-1.46)	1.34 (0.79-2.27)	1.28 (0.67-2.43)
Full cream milk	Intermediate + High	0.79 (0.39-1.60)	1.36 (0.61-3.03)	1.35 (0.50-3.64)
Semi-skimmed milk	Intermediate	0.78 (0.54-1.12)	1.00 (0.63-1.59)	1.04 (0.66-1.62)
	High	0.81 (0.61-1.08)	1.35 (0.96-1.91)	0.78 (0.54-1.12)
Butter	Intermediate + High	1.04 (0.55-1.96)	0.83 (0.36-1.89)	2.17 (0.99-4.75)
Margarine	Intermediate	0.94 (0.67-1.32)	1.46 (0.96-2.22)	0.80 (0.52-1.23)
	High	1.06 (0.81-1.38)	1.20 (0.84-1.70)	1.01 (0.71-1.42)

*House dust mite, cat dog, grass, birch or fungus.

†Milk or egg.

‡Multivariate model is adjusted for sex, maternal educational level, parental atopy, maternal smoking during pregnancy, smoking in the house at age 8 years, breast feeding, presence of older siblings, birth weight, overweight mother, allergic sibling, region and study arm.

§$p<0.05$

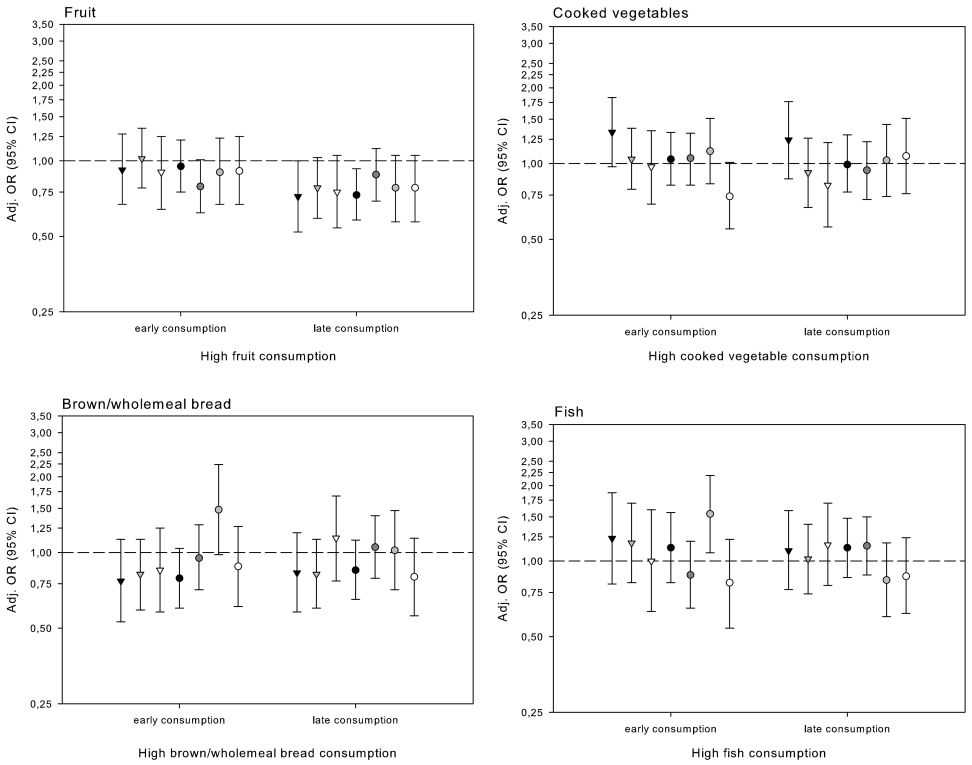

Figure 6.1: *Adjusted associations between high consumption of fruit, cooked vegetables, brown/wholemeal bread and fish at early and later age and wheeze (▼), dyspnea (▼), steroid use (▽), asthma symptoms (•), sensitisation to inhalant allergens (•), sensitisation to food allergens (•), and BHR (∘) at 8 years of age*

Figure 6.1 and figure 6.2 show the adjusted associations between high consumption at early age and later age and symptoms of asthma, atopy and BHR at 8 years of age. There were no associations between high cooked vegetable, brown/wholemeal bread, fish, full cream milk, semi-skimmed milk, butter and margarine consumption at either early or later age on symptoms of asthma or atopy, except for the occasional positive associations of high fish consumption at early age and sensitisation to food allergens, and high consumption of full cream milk at later age and wheeze. Consistent (borderline) beneficial associations for the different outcomes in 8-year-old children were observed for high fruit consumption at later age, but not for high consumption at early age. Beneficial associations for high fruit consumption at later age were statistically significant for wheeze and the composite variable 'asthma symptoms'.

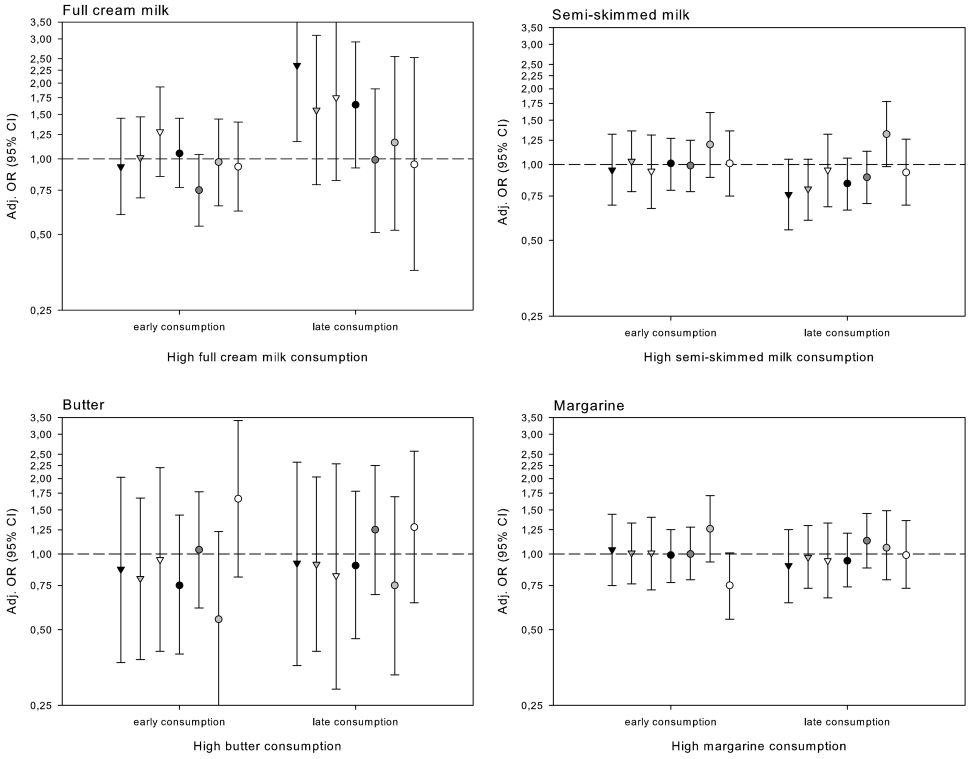

Figure 6.2: *Adjusted associations between high consumption of full cream milk, semi-skimmed milk, butter and margarine at early and later age and wheeze (▼), dyspnea (▼), steroid use (▽), asthma symptoms (•), sensitisation to inhalant allergens (•), sensitisation to food allergens (•), and BHR (◦) at 8 years of age*

6.4 Discussion

We have investigated associations between long-term dietary intake, and dietary intake at early and later age on symptoms and clinical outcomes of asthma and atopy at 8 years of age. No consistent associations were found for long-term dietary consumption or consumption at early or later age on outcomes at 8 years of age, except for fruit. Our results show that 'high' long-term fruit consumption is borderline beneficially associated with inhaled steroid use, 'asthma symptoms', and sensitisation to inhalant allergens. Furthermore, we observed beneficial associations of high fruit consumption at later age, but not at early age.

The associations between fruit consumption and childhood asthma and atopy observed in this study were not very strong. Results from previous studies in children are a bit ambiguous as well. Some studies have found beneficial associations between fruit consumption and lung function,[15] wheeze,[16] cough,[4,5] or rhinitis,[17] whereas

other studies did not find associations between fruit consumption and lung function,[18] asthma or wheeze,[7,19–21] or only found associations with specific types of fruit, especially the ones high in vitamin C.[3,8,22] High fruit consumption probably leads to a higher antioxidant status in the body, which provides more protection against oxidative damage through infections, passive smoking and air pollution.[23,24] Data on consumption of specific types of fruit were not available in our study. To understand more of the biological mechanisms underlying the associations between nutrition and asthma or allergy, more information about the intake of specific foods and nutrients is needed. If the effects observed in this study are caused by fruit-specific nutrients, estimating the intake of total fruit consumption has attenuated the effects on health outcomes.

The hypothesis that high fruit intake provides the lungs with more defence capacity against oxidative damage is probably the best explanation for the fact that we only observed associations between fruit consumption and respiratory symptoms assessed in the same time period. The hypothesis that nutrients which exert effects on airway development or Th-cell differentiation are especially important during early life[25] could not be confirmed in this study. We did not find associations between high fruit consumption at the age of 2 and 3 years on symptoms of asthma and atopy at 8 years of age. Probably, the time window of exposure relevant for effects on the developing airways and immune system is even earlier in life, before the age of two or during pregnancy. In this study we have chosen to investigate the effects of the child's diet from two years of age onwards. Evaluating dietary data assessed before the age of two would probably not be valid in this population because small age differences at this point in time still lead to quite distinctive dietary habits. Previous analyses in this cohort on the longitudinal effects of maternal fruit consumption during pregnancy revealed an overall beneficial effect on wheeze. However, the association lost statistical significance after adjustment for potential confounding factors.[26]

Maintenance of high fruit consumption over a longer period of time may reflect a more healthy diet or even a more healthy lifestyle in general. However, adjustment for socioeconomic and lifestyle factors like maternal education, maternal smoking during pregnancy, smoking in the home and breast feeding did not strongly alter the results. We also did not find consistent effects of 'high' long-term consumption of vegetables and fish, other foods that may reflect a healthy diet or lifestyle. However, since both socioeconomic status and a healthy lifestyle are complex concepts, residual confounding cannot be ruled out.

In this study there was some evidence for response bias, because children with complete data at 8 years of age included in the analyses probably were from a higher socioeconomic background (e.g. their mothers were more likely to have a higher educational level and to have breast fed their child, and less likely to have smoked during pregnancy or to be atopic). Furthermore, the overall stability of high fruit consumption from 2 to 8 years of age was higher in children of mothers with a higher educational level compared to children of mothers with an intermediate or low educational level.[27] Due to this type of bias, it is more likely that observed associations have been underestimated than overestimated. Improved ascertainment could have led to a higher proportion of symptomatic children with less consistent fruit con-

sumption, which would make the observed associations between 'high' long-term fruit consumption and health outcomes at 8 years of age stronger.

The use of self-reported dietary data could have led to misclassification of dietary exposure. However, it is unlikely that this has happened non-randomly. FFQ data are widely used in epidemiological studies and provide reasonably valid and reproducible estimates to rank individuals according to food group.[28] We have chosen a limited number of foods and food groups based on reported associations with asthma or atopy in previous studies. However, analyses of associations between several food groups and several outcomes could have led to chance findings due to multiple testing. Incidental associations for dietary consumption of a certain food and only one of the investigated outcomes must therefore be interpreted with caution.

The strength of our study was the use of longitudinal dietary data. Dietary exposure during childhood changes over time, which might change associations with symptoms of asthma or atopy as well. Longitudinal dietary data can be used to investigate differences of consumption at early or later age, providing more evidence on possible mechanisms of effects.

In 1997, Black and Sharpe[2] have proposed that changes in dietary fat intake during the last decades have contributed to the increase in asthma and atopic disease in children. They argued that the decrease in intake of n-3 polyunsaturated fatty acids from fish and saturated fats from butter and lard, and the increased intake of n-6 PUFAs from margarine and vegetable oils has led to an increased ratio of n-6 to n-3 fatty acid intake. This can result in increased production of arachidonic acid and prostaglandin E2 (PGE2) production with a consequent increase in the likelihood of atopic Th2 sensitisation, asthma and atopic disease.[2] Several studies have reported beneficial associations of dietary fish intake,[5,6,8,21,22,29–31] or harmful associations of margarine and vegetable oil intake[4,9,22,29,31,32] on asthma and atopic disease in children, whereas other studies did not find effects of n-3 PUFAs[4,7,19] or n-6 rich foods.[17] In this study there were no beneficial associations between high fish consumption during childhood and symptoms of asthma or atopy at 8 years of age. Neither did we find harmful effects of high margarine consumption. However, we have to acknowledge that the lack of finding an association with data on fish and margarine consumption might be due to the fact that the questions were not detailed enough to estimate the intake of n-3 or n-6 fatty acids or the ratio of these two. The effect mechanisms of dietary PUFA intake on inflammatory mediators and Th-cell differentiation are very complex, which makes it more likely that epidemiological studies produce conflicting results.[25]

An earlier study of the same cohort has reported beneficial associations of daily consumption of full cream milk and butter at two years of age on wheeze and asthma at three years of age.[7] The present study shows that this effect on these health outcomes does not last up to 8 years of age.

In conclusion, our findings suggest that 'high' long-term fruit consumption during childhood decreased the risk of inhaled steroid use, 'asthma symptoms' and atopic sensitisation. There was no strong evidence for different effects of timing of consumption of the foods investigated in this study. Future prospective studies could use more detailed dietary data to better assess nutrient or food specific effects.

6.5 References

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Chapter 7

General discussion

In this thesis, associations between maternal diet during pregnancy and childhood asthma and allergy were investigated in two large birth cohorts (SEATON and PIAMA). Furthermore, the stability of child's diet and associations between the child's diet and symptoms of asthma and atopy at 8 years of age were studied. The results of these studies add to a growing body of epidemiological evidence on the relation between nutrition and the development of allergic disease in childhood reviewed in chapter 2.

7.1 Main findings

In the SEATON birth cohort, we have found that consumption of apples and fish by the mother during pregnancy may have protective effects against the development of childhood asthma and allergic disease in 5-year-old children. However, there was no evidence for associations between maternal intake of the other investigated foods, such as total fruit, citrus/kiwi fruit, total vegetables, green leafy vegetables, fruit juice, whole grain products, fat from dairy products or butter versus margarine/low fat spread use during pregnancy and asthma, respiratory or allergic outcomes in the 5-year-old children.

Longitudinal analyses within the PIAMA birth cohort showed an increased risk of daily versus rare maternal consumption of nut products during pregnancy on childhood asthma outcomes. There were no associations between maternal vegetable, fish, egg, milk or milk products and nut consumption during pregnancy and longitudinal childhood outcomes.

Besides maternal diet during pregnancy, we have annually assessed childhood dietary habits within the PIAMA study. We have used this information to investigate tracking of the child's dietary habits from 2 to 8 years of age, and to investigate associations with asthma and allergy outcomes. Daily fruit and vegetable consumption decreased considerably with increasing age of the children. Highest tracking was seen for butter consumption. Tracking of fruit, cooked vegetables, fish, margarine and semi-skimmed milk consumption were relatively moderate. Yet, stability of dietary exposure is still fairly high compared to stability of other exposures of interest such as house dust allergen exposure over a time period of 8 years.[1]

No associations were found between early, late or long-term dietary consumption on symptoms of asthma or atopy at 8 years of age, except for fruit consumption. Long-term high fruit consumption was borderline inversely associated with steroid use, asthma symptoms, and sensitisation to inhalant allergens. We also observed a beneficial association between high fruit consumption at later age and asthma symptoms, but not between high fruit consumption at early age and asthma symptoms at 8 years of age.

7.2 Methodological considerations

7.2.1 Longitudinal analyses

From the review included in this thesis we can conclude that the current literature on the relation between nutrition and allergic disease in children is fragmentary, and hard to summarise in a systematic way. Most studies are of cross-sectional design or use cross-sectional statistical analyses to draw conclusions. The fact that the epidemiological evidence on the relation between nutrition and asthma or allergy is still inconsistent might have to do with temporality of associations. As we have seen in the results of the studies on the PIAMA birth cohort included in this thesis, the prevalence of certain dietary habits as well as the prevalence of symptoms of asthma or allergy change when the child grows older, which may lead to changes in associations over time as well. Most of the previous cross-sectional or prospective studies assessing the effect of diet (of the child or of the mother during pregnancy) on childhood asthma or allergy relate dietary data obtained at one point in time, to asthma or allergy outcomes at the same point in time or a (few) year(s) later. Timing of the assessment of exposure or outcomes is often different between studies, which makes their results difficult to compare. Longitudinal data could provide insight in temporality of associations, so when repeatedly measured dietary exposures and/or asthma/allergy outcomes are available, it is important to make use of them by conducting longitudinal statistical analyses. Repeated assessment of dietary intake provides better estimates of (changes in) dietary exposure during childhood.

Depending on the research question, several longitudinal statistical techniques are available. Generalised Estimating Equations (GEE) models are suitable to assess associations between exposure variables and the longitudinal development of outcome variables, taking into account that the repeatedly measured outcomes are correlated. Longitudinal tracking analyses can be used to assess the stability of repeatedly measured determinants.[2] Additionally, there are also longitudinal statistical techniques to model the temporal sequence of the relationship between repeatedly measured exposures and outcomes over a longer time period. The most basic of these models is the longitudinal time-lag model. In the time-lag model the exposure variables are modeled prior in time to the outcomes variables. The equation of the time-lag model for a dichotomous outcome variable Y is described in equation 7.1

$$\ln\left[\frac{P(Y_{it}=1)}{1-P(Y_{it}=1)}\right] = \beta_0 + \sum_{j=1}^{J}\beta_1 X_{ijt-1} + \ldots, \tag{7.1}$$

where Y_{it} is the outcome for subject i at time t, $P(Y_{it}=1)$ is the probability that the outcome at t_2 to t_m of subject i equals 1 (where m is the number of measurements and 1 means that subject i belongs to the group with a prevalent symptom), β_0 is the intercept, X_{ijt-1} is the predictor variable j for subject i at time $t-1$, β_1 is the regression coefficient for predictor variable j, and J is the number of predictor variables.[2]

In the study included in this thesis (chapter 6) we have chosen to investigate the association between food consumption at early age and later age on asthma outcomes

at 8 years of age. This was done because at 8 years of age additional information on clinical endpoints such as sensitisation and BHR were available and we wanted to investigate if the time window of exposure relevant for the development of asthma or allergy was in early age, or in later age, more closely prior to the asthma or allergy outcomes. The longitudinal time-lag model can be used to investigate associations between the annually assessed estimates of food consumption and subsequent asthma or allergy outcomes. In an earlier study of the PIAMA birth cohort, associations have been investigated between food consumption at two years of age and asthma symptoms at three years of age.[3] The importance of displaying associations between dietary exposure and asthma outcomes longitudinally is illustrated when we use the time-lag model to extend these previously reported associations over a longer period of time.

Figure 7.1 and figure 7.2 respectively show the longitudinal development of associations between daily versus non-daily brown/wholemeal bread consumption and daily versus rare full cream milk consumption at a previous measurement $(t-1)$ and wheeze at a subsequent measurement (t) from 3 to 8 years of age. From left to right these figures contain bars for the odds ratios and 95% confidence intervals between food consumption at age 2 and wheeze at age 3, food consumption at age 3 and wheeze at age 4, food consumption at age 4 and wheeze at age 5, and so forth. The most left bars in both figures are similar to the associations between brown/wholemeal bread or full cream milk and wheeze reported in the paper of Wijga and colleagues,[3] while the most right bars are comparable to the associations between brown/wholemeal bread or full cream milk consumption at later age (7 and 8 years of age) and wheeze at 8 years of age, reported in chapter 6 of this thesis.

Figure 7.1 shows that there were no large changes in associations between daily versus non-daily bread consumption and wheeze with increasing age, whereas figure 7.2 shows that daily versus rare full cream milk consumption at 2 years of age was inversely associated with wheeze at 3 years of age, but that daily versus rare full cream milk consumption at 7 years of age was positively associated with wheeze at 8 years of age.

This finding does not mean that the previously reported associations in the study of Wijga and colleagues[3] or chapter 6 of this thesis are not valid, but that longitudinal analyses are very useful to provide more insight in the development of associations, especially when exposure and outcomes change over time. There are several longitudinal statistical techniques which can be used to answer different types of research questions. The time-lag model illustrated above can be used to investigate differences in association between subsequent exposure and outcome measurements over a period of time.

7.2.2 Generalisability of the results

The initial population of 2000 pregnant women in the SEATON birth cohort were recruited during their first visit of the antenatal clinic of the Aberdeen Maternity Hospital (Aberdeen, UK). 1,924 singleton children were born to the 2,000 women, thirty-four women gave birth to twins, and 42 women lost their babies through mis-

Figure 7.1: *Adjusted associations between daily vs non-daily brown/wholemeal bread consumption at times $t-1$ and wheeze at times t (t = age in years) estimated by a longitudinal time-lag model.*

carriage, intrauterine death, stillbirth, or neonatal death. Follow-up of the original cohort was limited to these 1,924 singleton births. The 5,490 pregnant women attending the clinic for their first appointment during the recruitment period, who were not approached to participate in the study, were compared to the original 2000 women enrolled in the cohort. Study participants were slightly older, more likely to be primiparous, less likely to be current smokers, and, where ascertainable, more likely to be from non-manual social classes.[4] Follow-up took place when the children were 6 months, 12 months, 2 years and 5 years old. Comparison of responders and non-responders at 5 years of age demonstrated that the mothers who responded to the 5-yr questionnaire were less likely to smoke, were older, of higher socioeconomic status, less likely to have wheezed or to have had asthma, and were more likely to eat fruit, citrus/kiwi fruit, green leafy vegetables, whole grain products and oily fish, and to have a lower energy intake and a lower intake of fat from dairy products than non-responding mothers.

Participants of the PIAMA birth cohort were recruited from prenatal health care clinics in different regions in the Netherlands (north: Groningen, Leeuwarden and some surrounding municipalities, central: Utrecht, Amersfoort, Veenendaal and some surrounding municipalities and south-west: Rotterdam and some surrounding municipalities). Characteristics of the initial PIAMA participants have been compared with characteristics of the general Dutch population retrieved from data of Statistics Netherlands (CBS). PIAMA study participants were more likely to have a high education and less likely to have a low education, more likely to be from Dutch ethnic origin, and were less likely to smoke compared to the general Dutch population.[5]

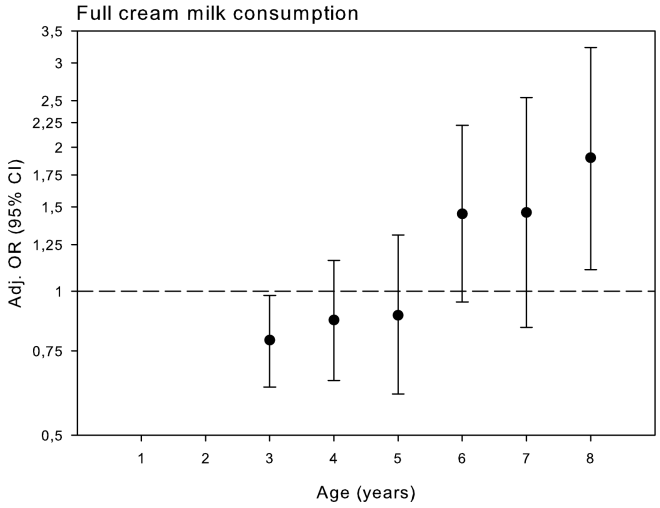

Figure 7.2: *Adjusted associations between daily vs rare full cream milk consumption at times $t-1$ and wheeze at times t (t = age in years) estimated by a longitudinal time-lag model.*

After 8 years of follow-up, children with complete data at 8 years of age were less likely to have a mother with atopy and a low educational level, a mother who smoked during pregnancy, or to be from region south-west compared to children at baseline. Furthermore, these children were more likely to have a mother with a high educational level, and had a higher mean birth weight compared to children at baseline.

These results suggest that the participants in both birth cohorts slightly over-represent participants with higher socioeconomic status and who are probably more health conscious. Therefore it is possible that the prevalences of dietary habits or health outcomes mentioned in this thesis may not apply to the general population of the UK or the Netherlands. Furthermore, the SEATON cohort consisted of women living, in or around Aberdeen in the north-east of Scotland, which is probably not a region that is representative for the whole UK.

7.2.3 Missing data

One of the main methodological problems of longitudinal studies is missing data. Participants can drop out towards the end of the study or skip a particular follow-up measurement. This leads to a situation in which not all subjects have complete information. The most often applied strategy to deal with missing data is simply to analyse the complete or available cases only. However, this strategy not only affects the precision of estimated associations, but can also cause bias because the mechanism in which missing data occur is often not completely at random.[6–8] It is known, for instance that subjects with a poorer socioeconomic status, or subjects

suffering from poor health during the follow-up period are more likely to drop out of longitudinal studies thus producing 'missing' values in a distinctly non-random way.[9] Multiple imputation (MI) is a sophisticated method that can be used to deal with missing data that is likely associated with the socioeconomic or lifestyle factors such as education, smoking or breastfeeding measured in the study.[6–8] When applying multiple imputation each missing value is replaced by several (m) simulated values. The differences between these datasets reflect the uncertainty of the missing values. Each of the m datasets is analysed in the same fashion by a complete method, which ignore the distinction between real and imputed values. The results, which may vary, are then combined by a simple arithmetic to obtain overall estimates and standard errors that reflect missing data uncertainty as well as finite-sample variation.[10] Within the PIAMA birth cohort study we have recently started to explore the application of this method to deal with missing values in our longitudinal data. The thesis of Scholtens contains examples of the application of MI on breastfeeding, overweight and asthma variables in the PIAMA study.[11] However, the attempt to apply MI to the longitudinal data included in this thesis proved to be very complicated, probably due to multicollinearity of the dietary data, and has not yet led to satisfying results.

7.2.4 The relation between nutrition and allergic disease

Studying the relation between nutrition and allergic disease is a major challenge for epidemiologists. First, precise and accurate assessment of dietary intake is intricate. Estimation of dietary intake encompasses the collection of information on the frequency and/or quantity of foods eaten, and calculation of the intake of food components (nutrients, energy) from food composition data. There are several methods to estimate dietary intake on individual level (e.g. food frequency questionnaire, 24h dietary recall, or dietary records) but none of them is completely perfect. All methods have their specific advantages but are also associated with random or systematic error such as reporting error, response bias, sampling bias and errors due to the use of food composition tables, food coding and portion size estimation, leading to misclassification of exposure.[12] The body's nutrient status not only depends on nutrient intake but also on absorption and metabolism. Measurement of biomarkers would be useful to validate dietary intake estimates and to capture the overall nutrient exposure and status more precisely, but should always be additional to other dietary exposure assessments.

Second, defining and measuring asthma and other allergic diseases is difficult. Young children are not yet able to perform spirometry or bronchial hyperresponsiveness tests, so diagnosis of asthma is often based on reported symptoms only. Asthma usually starts in early life but has different phenotypes with respect to its course and association with atopy. Wheezing in young children can be caused by respiratory infections and is often transient, whereas persistent wheezing is more likely to develop in children with atopy.[13] Prospective studies are needed to monitor the course of symptoms of allergic disease over time, and additional information on lung function and atopy is needed to form a more reliable diagnosis of asthma.

Third, it is very difficult to disentangle whether relations between nutrition and al-

lergic disease reflect effects of specific nutrients, specific foods or food groups, dietary patterns (e.g. Mediterranean diet) or a certain lifestyle (e.g. health-conscious or anthroposophic lifestyle or growing up on a farm). Nutrients and foods are often highly correlated with each other, making it difficult to identify independent effects. Diet in general is also related to other determinants such as overweight, socioeconomic status, and smoking. These multicollinearity and confounding phenomena make diet-disease associations difficult to interpret.[14] Furthermore, investigation of a relation between diet and allergic disease often involves multiple statistical comparisons, increasing the risk of chance findings. Conversely, existing associations can be missed due to misclassification of exposure, which tends to bias diet-disease associations towards the null.[14]

In the analyses conducted to investigate associations between diet and allergic disease in this thesis we have well-considered the influence of potential confounding factors, and corrected for them as comprehensively as possible. Several sensitivity analyses have been done to check if associations changed substantially when for instance different classifications of dietary consumption categories were made or when participants that possibly avoided consumption of certain foods due to allergy were excluded. We have been cautious with drawing strong conclusions when findings seemed incidental, e.g. when there was an association between intake of a certain food and only one of the investigated asthma or allergy symptoms, and considered the limitations of the study design when interpreting the results.

7.3 Conclusions and future perspectives

It has been hypothesised that early life (from conception to 2 years) dietary exposure might be particularly important in the development of childhood asthma, because the airways and immune system are developing during this period.[15] Recently, new epidemiological evidence on the association between maternal diet during pregnancy and childhood asthma and allergy from several birth cohort studies has become available, with promising results.[16–19] In this thesis, we have shown that maternal consumption of apples and fish during pregnancy may reduce the risk of children developing asthma or atopic disease, whereas maternal consumption of nut products may increase the risk of asthma. From this and the lack of strong consistent associations between long-term childhood diet and the child's diet at early or later age and asthma outcomes at 8 years of age, we may conclude that maternal diet during pregnancy possibly has a stronger influence on the development of allergic disease than the child's diet.

Current evidence on the relation between nutrition and allergic disease is inconsistent. Results from small cross-sectional studies are difficult to compare. Well-designed longitudinal multi-centre studies using standardized methods to assess dietary exposure and disease outcomes can provide more insight in the complex relation between nutrition and allergic disease. Underlying mechanisms can be possibly clarified further by conducting intervention and animal studies. If the results in this thesis are confirmed by these types of studies, recommendations on dietary modification during pregnancy may help to reduce the risk of developing asthma or allergy during

childhood.

Furthermore, we have shown that daily fruit and vegetable consumption decreased considerably with increasing age. According to the Netherlands Nutrition Centre (Voedingscentrum) children aged 4 to 12 should eat 150 to 200 grams of vegetables and 1.5 to 2 pieces of fruit a day.[20] In our study, we found that at 8 years of age more than 40% of the children did not daily eat fruit, whereas more than 60% of the children did not daily eat vegetables, which is worrisome. Since our study population is likely to be of higher socioeconomic status and more health conscious, the true picture in the general population of children might be even worse. Possible public health interventions in the form of dietary modifications would therefore not solely be beneficial to reduce the risk of childhood asthma and allergy but would also have other health benefits.

7.4 References

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Chapter 8

Summary/Samenvatting

Summary

Since the 1960's of the last century the prevalence of asthma and allergic disease has increased considerably. Although asthma and allergy have genetic determinants, the observed increase has occurred too rapidly for genetic changes to explain the increase. This, and the fact that the prevalence of asthma and allergy is much higher in the more affluent Westernized societies than in rural or developing regions, has led to consideration of several environmental and lifestyle determinants to explain the increase. One of these determinants is diet. Increasing prosperity in the western world has led to large changes in diet. Reduced antioxidant intake and altered fatty acid intake ratio have been related to an increased risk to develop asthma or allergy. Numerous epidemiological studies have investigated the relation between nutrition and allergic disease. The review in **chapter 2** of this thesis gives an elaborate overview of the available literature to date. However, from this review we can conclude that the evidence for the relation between diet and allergic disease in children is still inconsistent.

The early onset of asthma and its persistence has recently led to considerable interest in the effects of dietary exposure in fetal life, a critical period for the development of the airways and immune system. The Study of Eczema and Asthma To Observe the influence of Nutrition (SEATON) birth cohort in Aberdeen in the UK was set up to investigate associations between maternal diet during pregnancy and childhood asthma and allergic disease. 2,000 healthy pregnant women have been recruited during their first visit of the Aberdeen Maternity Hospital's antenatal clinic. Maternal diet during pregnancy was assessed by a large food frequency questionnaire, and the 1,924 children born in this cohort have been followed up for 5 years. Most of the previous reports relating maternal diet during pregnancy to childhood asthma and atopy have focused on individual nutrients. In the study included in **chapter 3** of this thesis, we were one of the first to investigate the influence of maternal intake of specific foods during pregnancy and the subsequent development of childhood asthma and atopic disease. We have reported beneficial associations between maternal apple intake and childhood wheeze and asthma, and between maternal fish intake and childhood eczema and hay fever in 5-year-old children. However, there was no evidence of associations between asthma, respiratory or atopic outcomes in 5-year-old children and maternal intakes of total fruit, citrus/kiwi fruit, total vegetables, green leafy vegetables, fruit juice, whole grain products, fat from dairy products or butter versus margarine/low fat spread use.

In **chapter 4** we have investigated associations between maternal diet during pregnancy and childhood asthma symptoms longitudinally within the PIAMA birth cohort study. The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study, initiated in 1996, consisted of a natural history part to study the role of environmental and dietary risk factors for the development of asthma and allergy in childhood, and of an intervention part to evaluate the use of mite-impermeable mattress and pillow covers. 4,146 pregnant women were enrolled (of whom 1,327 were atopic and 2,819 were non-atopic) in the study, their 3,963 newborn children were annually followed up until 8 years of age. The questionnaire adminis-

tered to the pregnant women contained some questions about their diet during the last three months. We have used longitudinal Generalized Estimating Equations (GEE) to assess associations between maternal dietary consumption during pregnancy to childhood symptoms of asthma from 1 to 8 years of age simultaneously. Our results showed no consistent associations between the maternal intake of the investigated food groups during pregnancy and childhood asthma symptoms until the age of 8, except for nut products. Daily versus rare nut product consumption during pregnancy was consistently significantly positively associated with childhood wheeze, wheeze without cold, dyspnea, dyspnea without cold, inhaled steroid use, doctor-diagnosed asthma and the composite variable 'asthma symptoms'.

The PIAMA birth cohort study has annually assessed dietary habits until 8 years of age. In **chapter 5** of this thesis, this information is used to investigate the development of dietary habits from early to later age by tracking analyses. Most previous studies on the relation between nutrition and health in children have usually measured dietary consumption cross-sectionally. It is not completely clear how well measurements at one point in time reflect nutrition over a longer period. Tracking analyses can be used to assess stability of dietary habits over time. If dietary habits 'track' very well, then dietary exposure measured at some point in time is possibly valid for a longer period during childhood, which makes the assessment of associations with health outcomes less complex. We have found that daily fruit and vegetable consumption decreased considerably over time. Highest tracking was seen for butter consumption. Tracking of fruit, cooked vegetables, fish, margarine and semi-skimmed milk consumption were relatively moderate to low. Tracking of fruit, fish and margarine consumption significantly differed by maternal educational level. We can conclude from these results that the assumption that dietary consumption estimated at a certain time point reflects the habitual intake during childhood is not valid for all foods.

The hypothesis that early life dietary exposure might be particularly important in the development of childhood asthma raised the question whether the child's diet at early age (2 and 3 years of age) was stronger associated with symptoms and clinical outcomes of asthma or atopy at 8 years of age than the child's diet at later age (7 and 8 years of age). Furthermore we were interested in the effects of long-term dietary habits on childhood asthma or allergy. The results presented in **chapter 6** show no associations between early, late or long-term dietary consumption on symptoms of asthma or atopy at 8 years of age, except for fruit. Long-term high fruit consumption was borderline inversely associated with inhaled steroid use, asthma symptoms, and sensitisation to inhalant allergens. Furthermore we observed a beneficial association between high fruit consumption and asthma symptoms at later age, but not at early age.

In **chapter 7**, the main findings of this thesis are discussed. Additionally, we address some methodological issues and make some recommendation for future research. From the current literature and the results presented in this thesis, we may conclude that maternal diet during pregnancy possibly has a stronger influence on the development of allergic disease than the child's diet. Well-designed longitudinal multi-centre studies using standardized methods to assess dietary exposure and dis-

ease outcomes can provide more insight in the complex relation between nutrition and allergic disease. Underlying mechanisms can be possibly clarified further by conducting intervention and animal studies. If the results in this thesis are confirmed by these types of studies, recommendations on dietary modification during pregnancy may help to reduce the risk of developing asthma or allergy during childhood.

Samenvatting

De prevalentie van astma en allergische aandoeningen is sterk toegenomen sinds de jaren 60 van de vorige eeuw. Astma en allergie hebben een sterke genetische component, maar omdat de stijging in prevalentie zo snel heeft plaatsgevonden, is het waarschijnlijk dat ook omgevings- en leefstijlfactoren een rol spelen in de ontwikkeling van astma en allergie. Het feit dat de prevalentie van astma hoger is in meer welvarende Westerse landen dan in plattelandsgebieden of ontwikkelingslanden heeft ertoe geleid dat verschillende omgevings- en leefstijlfactoren in beschouwing worden genomen om de stijging in prevalentie te verklaren. Een van deze leefstijlfactoren is voeding. De toegenomen welvaart in de Westerse wereld heeft tot grote veranderingen in het voedingspatroon geleid. De afgenomen inname van antioxidanten en een veranderd patroon van vetzuurinname zijn mogelijk gerelateerd aan het toegenomen risico op het ontwikkelen van astma of allergie.

De relatie tussen voeding en allergische aandoeningen is in een groot aantal epidemiologische studies onderzocht. Het overzichtsartikel in **hoofdstuk 2** van dit proefschrift geeft een uitgebreid beeld van de beschikbare literatuur tot nu toe. Uit dit overzichtsartikel kunnen we echter concluderen dat het bewijs voor de relatie tussen voeding en allergische aandoeningen nog steeds inconsistent is.

Het ontstaan van astma en allergie op relatief vroege leeftijd heeft recentelijk geleid tot een toegenomen interesse in de invloed van voeding in het vroege leven (vanaf de conceptie tot aan ongeveer tweejarige leeftijd), wanneer de luchtwegen en het immuunsysteem in ontwikkeling zijn. Het geboortecohort Study of Eczema and Asthma To Observe the influence of Nutrition (SEATON), in Aberdeen in Schotland is opgezet om deze relatie te bestuderen. Voor dit onderzoek zijn 2000 gezonde zwangere vrouwen gerekruteerd tijdens hun eerste bezoek aan de verloskundige kliniek van het Aberdeen Maternity Hospital. Het voedingspatroon van de aanstaande moeders werd vastgesteld door middel van een uitgebreide voedselfrequentievragenlijst afgenomen rondom de 32e week van de zwangerschap. De 1924 kinderen die zijn geboren binnen dit cohort zijn opgevolgd tot de leeftijd van 5 jaar. Eerdere onderzoeken naar voeding van moeders tijdens de zwangerschap en astma en atopie bij hun kinderen hebben zich vooral gericht op individuele nutriënten. De studie in **hoofdstuk 3** van dit proefschrift is de eerste die zich richt op de invloed van specifieke voedingsmiddelen. In deze studie hebben we gevonden dat kinderen van moeders die meer dan 4 appels per week aten een significant lager risico op piepen op de borst en astma hadden vergeleken met kinderen van moeders die minder dan 1 appel per week aten. Ook hebben we gevonden dat kinderen van moeders die minstens 1 keer per week vis of vette vis aten een significant lager risico op respectievelijk eczeem of hooikoorts hadden vergeleken met kinderen van moeders die nooit vis of vette vis aten. Er waren geen aanwijzingen voor associaties tussen consumptie van fruit, citrusvruchten of kiwi, groente, groene bladgroente, vruchtensap, volkorenproducten, vet uit zuivelproducten of boter versus margarine/halvarine gebruik en astma, respiratoire of atopische klachten bij de 5-jarige kinderen.

In **hoofdstuk 4** hebben we de relatie tussen voeding van de moeder tijdens de zwangerschap en symptomen van astma bij kinderen in de PIAMA geboortecohortstu-

die longitudinaal onderzocht. De Preventie en Incidentie van Astma en Mijt Allergie (PIAMA) geboortecohortstudie, geïnitieerd in 1996, bestaat uit een natuurlijk beloop en een interventiegedeelte. De natuurlijk beloop studie is opgezet om de rol van omgevingsfactoren en voeding in de ontwikkeling van astma en allergie te bestuderen, terwijl het interventiegedeelte is opgezet om de effectiviteit van mijt-ondoorlaatbare matras- en kussenhoezen te bestuderen. In de PIAMA studie zijn 4146 zwangere vrouwen geïncludeerd van wie 1327 atopisch en 2819 niet atopisch. De 3963 kinderen geboren binnen dit cohort zijn jaarlijks opgevolgd tot en met de leeftijd van 8 jaar. De vragenlijst afgenomen tijdens de zwangerschap bevatte een aantal vragen over voedingsgewoonten van de aanstaande moeders in de afgelopen drie maanden. We hebben de longitudinale analysetechniek Generalised Estimating Equations (GEE) gebruikt om associaties tussen voeding tijdens de zwangerschap en symptomen van astma bij de kinderen vanaf 1 tot 8-jarige leeftijd te berekenen. De resultaten lieten geen consistente associaties zien tussen consumptie van de onderzochte voedingsmiddelen (fruit, groente, vis, melk, melkproducten, noten en notenproducten zoals pindakaas) en symptomen van astma bij de kinderen, behalve voor consumptie van notenproducten zoals pindakaas. Kinderen van moeders die dagelijks notenproducten hadden geconsumeerd tijdens de zwangerschap hadden een consistent verhoogd risico op piepen op de borst, kortademigheid, het gebruik van inhalatiecorticosteroïden, en astma gediagnosticeerd door een arts van leeftijd 1 tot en met 8 jaar vergeleken met kinderen van moeders die zelden of nooit notenproducten hadden geconsumeerd tijdens de zwangerschap.

In de PIAMA geboortecohortstudie is tijdens de follow-up jaarlijks naar voedingsgewoontes van de kinderen gevraagd. In **hoofdstuk 5** van dit proefschrift is deze informatie gebruikt om de ontwikkeling van de voedingsgewoontes over de tijd te volgen door middel van longitudinale 'tracking' analyses. Eerdere studies naar de relatie tussen voeding en gezondheid bij kinderen zijn voornamelijk dwarsdoorsnede onderzoeken, wat betekent dat voedingsgewoonten maar op één bepaald tijdstip zijn nagevraagd. Het is nog niet duidelijk of voedingsgewoonten van kinderen gemeten op één bepaald tijdstip representatief zijn voor voedingsgewoonten over een langere tijdsperiode. Tracking analyses kunnen gebruikt worden om de stabiliteit van voedingsgewoonten over een langere tijdsperiode vast te stellen. Wanneer voedingsgewoonten relatief stabiel zijn over de tijd is het minder lastig om eventuele relaties met gezondheidsuitkomsten vast te stellen. Uit deze studie kwam naar voren dat het percentage kinderen dat dagelijks fruit en groente eet aanzienlijk afneemt naarmate zij ouder worden. De hoogste tracking werd gezien voor dagelijkse consumptie van boter. De tracking van dagelijkse consumptie van fruit, gekookte groente, en halfvolle melk en de regelmatige consumptie van vis was relatief gematigd tot laag. De tracking van vis en margarine consumptie was mede afhankelijk van het opleidingsniveau van de moeder. Uit deze studie kunnen we concluderen dat cross-sectioneel gemeten voedingsgewoonten op jonge leeftijd niet altijd representatief zijn voor voedingsgewoonten over een langere tijdsperiode.

De hypothese dat voeding voornamelijk vroeg in het leven het risico op het ontstaan van astma bepaald, leidde tot de vraag of bepaalde voedingsgewoonten van het kind op vroege leeftijd (2 en 3 jaar) sterker geassocieerd zijn met symptomen van

astma of allergie dan voedingsgewoonten van het kind op latere leeftijd (7 en 8 jaar). Ook waren we geïnteresseerd of een consistent voedingspatroon vanaf leeftijd 2 tot en met 8 jaar geassocieerd was met astma en allergie op 8-jarige leeftijd. De resultaten van dit onderzoek zijn gepresenteerd in **hoofdstuk 6**, en laten zien dat er geen associaties te zien waren tussen de voeding van het kind gemeten op vroege leeftijd, latere leeftijd of een consistent voedingspatroon over een langere tijdsperiode en symptomen van astma op 8-jarige leeftijd. Een uitzondering was fruitconsumptie. Kinderen die consistent dagelijks fruit aten vanaf leeftijd 2 tot en met 8 jaar hadden een lager risico op het gebruik van inhalatiecorticosteroïden, astmasymptomen en sensibilisatie voor inhalatie-allergenen op 8-jarige leeftijd vergeleken met kinderen die niet consistent dagelijks fruit aten. Deze associaties waren echter net niet statistisch significant op 95%-niveau. De associaties met astma symptomen werd ook gezien voor dagelijkse fruitconsumptie op 7- en 8-jarige leeftijd, maar niet voor dagelijkse fruitconsumptie op 2- en 3-jarige leeftijd.

In **hoofdstuk 7** worden de belangrijkste bevindingen van dit proefschrift bediscussieerd. Daarnaast worden er een aantal methodologische aspecten behandeld en worden er aanbevelingen voor verder onderzoek gedaan. Uit de huidige literatuur en het onderzoek gepresenteerd in dit proefschrift kunnen we concluderen dat voeding tijdens de zwangerschap mogelijk een grotere invloed heeft op het ontstaan van astma en allergie bij kinderen dan voeding van het kind zelf. Longitudinale studies met gestandaardiseerde methoden kunnen mogelijk meer inzicht verschaffen in de complexe relatie tussen voeding en allergische aandoeningen. Onderliggende mechanismen zouden mogelijk verder onderzocht kunnen worden door middel van interventie en/of dierstudies. Wanneer de resultaten in dit proefschrift ondersteund worden door dit soort studies dan kunnen er mogelijk voedingsadviezen worden aangereikt om het risico op astma en allergie bij kinderen te verlagen.

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About the author

Saskia Mirjam Willers was born in The Hague, The Netherlands on the 7^{th} of January 1980. She completed secondary school in 1998 at the Raayland College in Venray and started her study Environmental Health Sciences with complementary MSc courses in Epidemiology at Maastricht University (The Netherlands). After her internship at the Institute for Risk Assessment Sciences (IRAS) at Utrecht University (The Netherlands) on gas cooking, kitchen ventilation and asthma, allergic symptoms and sensitisation within the PIAMA birth cohort study, she received her MSc degree in Environmental Health Sciences in 2003. In 2003/2004 she followed additional MSc courses in Health Economics, Policy and Law at Erasmus University Rotterdam (The Netherlands). From July 2004 she was appointed as junior researcher and PhD fellow at IRAS to work on analyses of exposure data on different combustion products in kitchens of participants' homes in the PIAMA study. In addition, she was involved in analyses of pooled data on PM_{10} and lung function in children within the PATY study, and on a literature review on nutrition and allergic disease within the Global Allergy and Asthma European Network (GA^2LEN) work package Nutrition. In 2006 she was awarded a GA^2LEN exchange fellowship to spend 5 months at the Department of Environmental and Occupational Medicine at the University of Aberdeen in Scotland to investigate associations between maternal diet during pregnancy and asthma, respiratory and atopic outcomes in 5-year-old children within the SEATON birth cohort study. When she returned to IRAS she continued working on the completion of this thesis. From the 1^{st} of October 2008 she will be appointed as a postdoctoral fellow at the Institute of Environmental Medicine at Karolinska Institutet in Stockholm, Sweden.