THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Early dietary exposure is associated with allergy development in the FARMFLORA birth cohort

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EARLY DIETARY EXPOSURE IS ASSOCIATED WITH ALLERGY DEVELOPMENT IN THE FARMFLORA BIRTH COHORT
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Front cover: Illustration of the FARMFLORA birth cohort.

EARLY DIETARY EXPOSURE IS ASSOCIATED WITH ALLERGY DEVELOPMENT IN THE FARMFLORA BIRTH COHORT

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ABSTRACT

The prevalence of allergy is markedly low in children who grow up on farms. Contact with livestock and consumption of unpasteurized milk have been associated with allergy protection, although other elements in the environment may contribute. The FARMFLORA birth cohort was established to identify factors that may be part of the allergy protection of the farming environment. In this thesis, early dietary exposures of farm and control children were evaluated in relation to allergy at three years of age, including: 1) maternal diet during pregnancy and lactation; 2) fatty acid composition of infant sera at birth and in sera and breast milk four months postpartum; 3) introduction practices of complementary foods; and 4) diet at one year of age. Twenty-eight children from dairy farms in southwestern Sweden and 37 non-farm control children from the same rural area were included in the cohort. The children were examined clinically by pediatricians to diagnose food allergy, eczema, asthma and rhinitis.

Farming mothers consumed more full-fat dairy and saturated fats during pregnancy and lactation than control mothers, who instead consumed more margarines and oils. The same pattern was found in the children's diet at one year of age. The higher intake of saturated fats among the farming mothers was reflected in their breast milk as higher proportions of saturated fatty acids and lower proportions of the polyunsaturated fatty acids linoleic and alpha-linolenic acid. However, the only difference found in the infants' sera was higher proportions of arachidonic acid at birth and lower proportions of the monounsaturated omega-7 fatty acid 18:1 four months postpartum in farmers' children. Neither did the timing of the introduction of complementary foods differ between farm and control children, except for an earlier introduction of nuts in farm children.

One farm child (4%) and ten control children (25%) were allergic by the age of three years. The intake of margarines and oils both by the mothers during pregnancy and lactation and by the children at one year of age was weakly associated with allergy development at three years of age. Higher intake of pork was also found in subsequently allergic as compared to healthy children when farmers were excluded from the analysis. The most pronounced difference between healthy and subsequently allergic children was higher proportions of the long-chain omega-3 polyunsaturated fatty acid eicosapentaenoic acid in serum, both at birth and four months postpartum, reflecting maternal fish intake during pregnancy and lactation. Concordantly, a pattern of earlier introduction of fish in the healthy children was observed, together with higher intake of seafood at one year of age. There was also a tendency among healthy children that flour and eggs were introduced earlier. Exclusive breastfeeding was associated with less allergy, although the protective effect was only observed for breastfeeding up to three months of age.

In conclusion, a low margarine consumption by the mother and child was weakly associated with less allergy as well as with growing up on a farm. Consumption of fish by mothers during pregnancy and lactation as well as in the early diet of children was associated with a decreased risk of allergy development, although unrelated to farm residence. Tendencies of late introduction of complementary foods were related to an increased risk of allergy development.

LIST OF PUBLICATIONS

This doctoral thesis is based on the work contained in five papers:

Paper I: Karin Jonsson, Malin Barman, Sara Moberg, Agneta Sjöberg, Hilde K. Brekke, Bill Hesselmar, Susanne Johansen, Agnes E. Wold and Ann-Sofie Sandberg. **Fat intake and breast milk fatty acids composition in farming and nonfarming women and allergy development in the offspring.** *Pediatric Research*, 2016. **79**(1): p. 114-123.

Paper II: Karin Jonsson, Malin Barman, Sara Moberg, Agneta Sjöberg, Hilde K Brekke, Bill Hesselmar, Ann-Sofie Sandberg and Agnes E. Wold. **Serum fatty acids in infants, reflecting maternal fish consumption, were inversely associated with allergy development but not related to farm residence.** *Acta Paediatrica,* 2016. **105** (12): doi: 10.1111/apa. 13592.

Paper III: Malin Barman, Karin Jonsson, Agnes E. Wold and Ann-Sofie Sandberg. Low proportions of eicosapentaenoic acid in cord serum are associated with allergy development: results from the FARMFLORA birth cohort. *Manuscript.*

Paper IV: Karin Jonsson, My Green, Malin Barman, Agneta Sjöberg, Hilde K Brekke, Agnes E. Wold and Ann-Sofie Sandberg. **Diet in 1-year-old farm and control children and allergy development: results from the FARMFLORA birth cohort.** *Food and Nutrition Research*, 2016. **60**: p. 32721-32734.

Paper V: Karin Jonsson, Malin Barman, Hilde K. Brekke, Bill Hesselmar, Ann-Sofie Sandberg and Agnes E. Wold. **Introduction of complementary foods and allergy development in farm and nonfarm children.** *Manuscript.*

Papers not included in this thesis:

Malin Barman, Karin Jonsson, Anna Sandin, Agnes E. Wold and Ann-Sofie Sandberg. **Serum fatty acid profile does not reflect seafood intake in adolescents with atopic eczema**. *Acta Paediatrica,* 2014. **103**(9): p. 968-976.

Malin Barman, Karin Jonsson, Bill Hesselmar, Anna Sandin, Ann-Sofie Sandberg, Agnes E. Wold. **No association between allergy and current 45-hydroxy vitamin D in serum or vitamin D intake.** *Acta Paediatrica*, 2015. **104**(4): p. 405-413.

CONTRIBUTION REPORT

Paper I: The author, Karin Jonsson (KJ), performed all analyses of the nutritional data, performed statistical calculations, was involved in the interpretation of the data and was responsible for writing the manuscript.

Paper II: KJ performed preceding analyses of the nutritional data, performed statistical calculations, was involved in the interpretation of the data and was responsible for writing the manuscript.

Paper III: KJ performed preceding analyses of the nutritional data, was involved in the interpretation of the data and reviewed and revised the manuscript.

Paper IV: KJ performed statistical calculations, was involved in the interpretation of the data and was responsible for writing the manuscript.

Paper V: KJ performed statistical calculations, was involved in the interpretation of the data and was responsible for writing the manuscript.

ERRATA

**Page 27.** The section *Fatty acid analysis in breast milk and serum* should be:

Fatty acid analysis in breast milk and sera
Fatty acids in sera collected four months postpartum were extracted with chloroform and methanol according to Lee et al. (120) and the phospholipid fraction was separated with solid phase extraction on aminopropyl solid phase extraction columns (121) before methylation (122). Fatty acids in cord sera and breast milk were analyzed with direct transesterification (122) without prior fat extraction. After methylation […] see Papers I, II and III.

Page 31. Low fat milk should be Full-fat milk and vice versa in *Figure 5*.
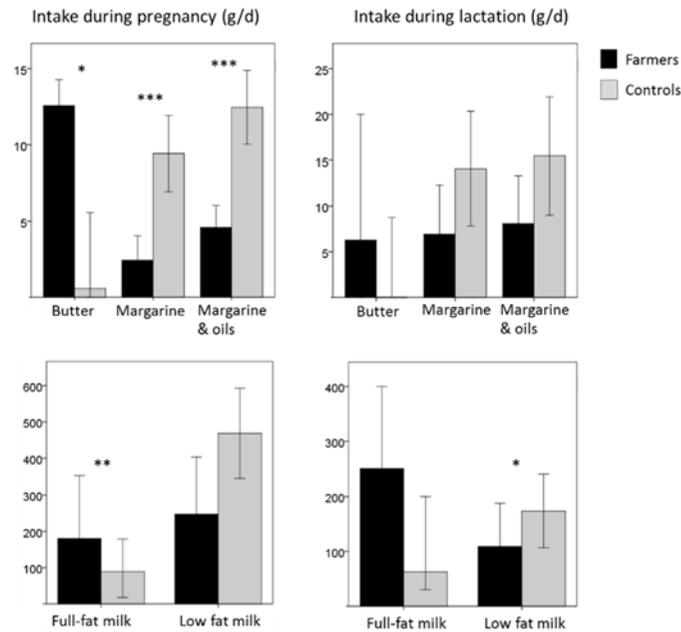
Page 32. Linolenic acid should be Linoleic acid in *Figure 6*.
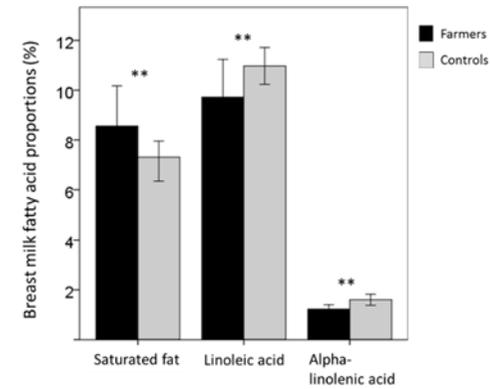
Page 37. Farmers and Controls should be Healthy and Allergic in the legend of *Figure 8*.
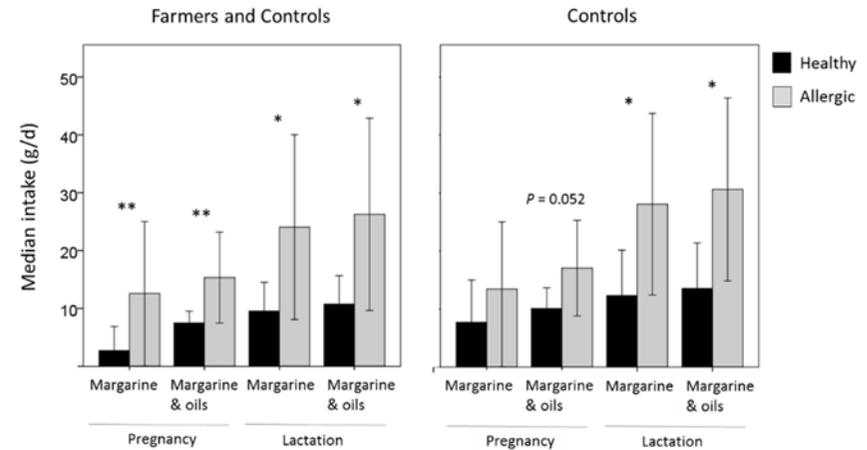
Page 41. In *Table 10,* it should be the following for introduction of fish and eggs ≤10 months:

Fish: 100% healthy and 82% allergic (all subjects), 100% healthy and 90% allergic (controls).
Eggs: 96% healthy and 64% allergic (all subjects), 96% healthy and 60% allergic (controls).

TABLE OF CONTENTS

INTRODUCTION

The prevalence of allergy has increased drastically during the second half of the 20th century (1, 2). According to Miljöhälsorapporten 2013, 16% of four-year-old children in Sweden had asthma or hayfever and seven and six percent had eczema in creases or on hands, respectively (3).

Allergies are a result of a disturbed immune system, causing our bodies to react to nonpathogenic proteins that are tolerated by individuals with a healthy immune system (4). There are to date no cures or effective measures of prevention and the reasons behind the allergy increase are not known, although some hypotheses have been proposed. In 1989, Strachan formulated the 'Hygiene Hypothesis', in which a decrease in family size, especially in terms of older siblings and higher hygienic standards, were suggested as possible explanations of the increase in allergy (5). The hygiene hypothesis was further evolved by Wold in 1998, who suggested that the commensal intestinal flora might be central in the process of immune maturation (6). In line with this hypothesis, Paolo Matricardi showed that infections spread via the fecal-oral route were protective, whereas airborne viral infections were not, pointing to the importance of microbial stimulation via the gut for protection against allergy (7). Further, a high complexity of the gut microbiota in early infancy has been associated with a lower risk of allergy development (8).

Already in the 1870s, Blackley predicted that hay fever would become more common in the future as civilization and education advanced (9). Blackley had observed that '…the persons who are most subjected to the action of pollen belong to a class which furnishes the fewest cases of the disorder, namely, the farming class…' (9). In line with these observations, an inverse association between allergy development and early exposure of a farming environment have been found in a large number of studies (10). Contact with livestock and feed, and consumption of unpasteurized farm milk have been proposed to explain this 'farm protection', although parts of the protection are left unexplained.

The diet of farmers has been evaluated in a few studies that consistently showed that farmers consumed more full-fat dairy products compared to non-farming control subjects (11-14). Consumption of butter and dairy products has been negatively associated with allergy in a number of studies (15-17), while margarine intake instead has been positively associated with allergy (18, 19). Margarines are rich in vegetable oils, including fatty acids from the omega (n)-6 family, and, in an earlier study in 1997, Black and Sharpe observed that the increase in the development of allergy in the past decades took place in parallel with an increase in the use of margarines and vegetable oils (20). Hence, the authors hypothesized that an increase in n-6 and a decrease in long-chain n-3 polyunsaturated fatty acids were causative factors in the development of allergy (20). Concordantly, the intake of fish, which is our main source of long-chain omega-3 fatty acids, has repeatedly been associated with less allergy (21, 22).

The protection observed from growing up on farms seems to lie well in agreement with the hygiene hypothesis. This is investigated further in the FARMFLORA birth cohort, in which a large number of bacteriological and immunological samples have been collected from children raised on dairy farms and from children living in rural areas who were not raised on farms. This part of the project is performed at Clinical Bacteriology, Sahlgrenska Academy. The role of the diet in the protective farming environment has been evaluated less extensively in previous studies. In the FARMFLORA birth cohort, information about nutritional exposures of the infants, already in utero, have been collected up to three years of age. The aim of this thesis is to investigate whether the diet of farmers may be part of the action of protection from allergy in the farming environment.

OBJECTIVES

The overall aim of this thesis was to investigate whether the early diet in dairy farmers' children, as well as their mothers' diet during pregnancy and lactation, could be part of the allergy protection afforded by growing up in a farming milieu. Differences between farming and non-farming control families were studied, and differences between healthy and subsequently allergic children were analyzed. An overview of the five papers included in the thesis are found in figure 1.

Specific research questions in the thesis were:

- Is there a difference in maternal diet during pregnancy and lactation between farmers and controls and between mothers whose children develop or do not develop allergy? (Paper I)

- Does fatty acid composition of breast milk and infant sera at birth and at four months postpartum differ between farmers and controls, and between children who developing or do not develop allergy? (Papers I, II and III)

- Do the proportions of different fatty acids in infant sera correlate with the proportions of the corresponding fatty acids in breast milk, and do breast milk proportions in turn correlate with maternal diet during pregnancy and lactation? (Paper II)

- Does the child's diet at one year of age differ between farm and control children, and between children who develop or do not develop allergy? (Paper IV)

- Does the dietary pattern of one-year-old farm children resemble the dietary pattern of their mothers during pregnancy and lactation? (Paper IV)

- Does the introduction of complementary foods differ between farm and control children, and between children who develop or do not develop allergy? (Paper V)
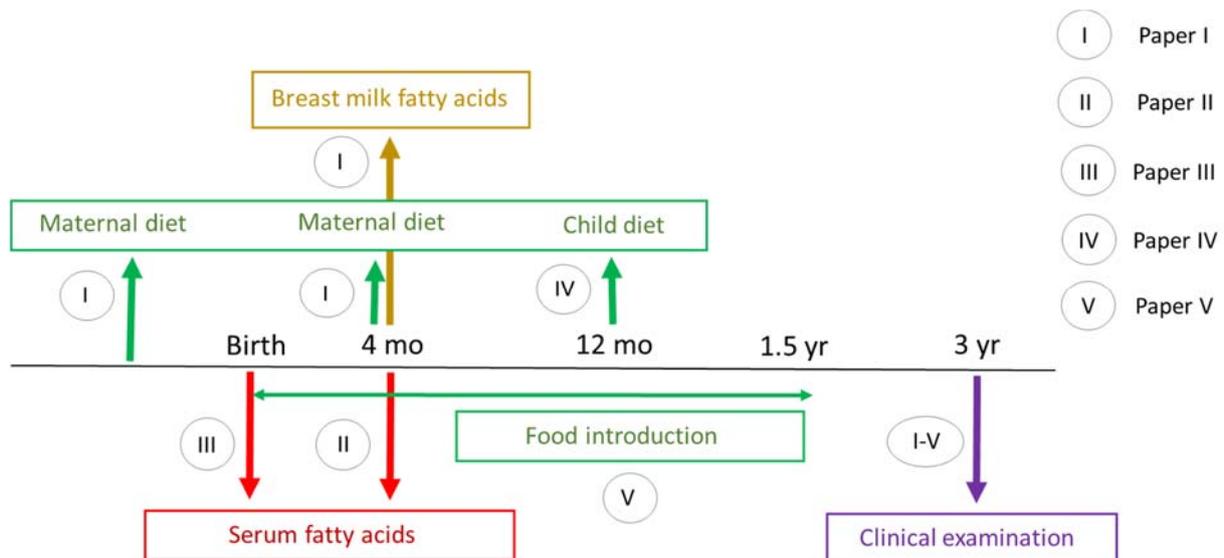

Figure 1. Outline of the thesis with an overview of the five papers included.

LITERATURE OVERVIEW

THE IMMUNE SYSTEM AND ALLERGY ONSET

Allergy denotes immune mediated hypersensitivity. Different immune mechanisms may be involved in different types of allergies. In this thesis, dietary and life-style factors that affect the risk of developing IgE-mediated allergy, also termed atopic allergy, are evaluated. IgE-mediated allergy is caused by the production of IgE antibodies by the individual as a reaction against naturally occurring environmental substances, termed allergens. In IgE-mediated allergy, the allergens are almost always proteins. The atopic individual commonly reacts to proteins in the food, e.g. milk, egg or fish proteins, or in the air (pollen proteins from birch and grass, or proteins present in dander from cats, horses and other companion animals). The IgE antibody is unique in its capacity to bind to mast cells. Mast cells are located in the connective tissue under the skin and mucosal surfaces, usually in close connection to blood vessels.

The allergic reaction

An allergic reaction is initiated by the cross-linking by an allergen of at least two allergen specific IgE antibodies bound to the surface of a mast cell (Figure 2, right panel). The cross-linking activates the mast cell and causes the cell to degranulate, releasing powerful mediators, such as histamine. The histamine causes endothelial cells of blood vessels nearby to contract, resulting in a leakage of blood plasma into the tissue. The blood vessels are also dilated, which makes the blood flow slower and more turbulent (Figure 2, right panel) (4).

This initial hypersensitivity reaction continues to a more persistent reaction that may go on for several hours, with increased swelling. The transition is mediated by leukotrienes, which are formed from arachidonic acid that is cleaved off from membrane phospholipids inside an activated mast cell. The action of the leukotrienes on the blood vessel are similar to that of histamine, but more powerful and persisting (4)

In a last step, cytokines produced by mast cells, macrophages and activated T cells attract inflammatory cells from the blood circulation, mainly eosinophilic granulocytes and T cells, the latter of the so called Th2 type. Th2 cells participate in the allergic reaction by producing cytokines such as IL-4, IL-5, IL-9 and IL-13 that are involved in, e.g., activation of eosinophils and increased production of eosinophils in the bone marrow, increased production of mucus in the airways and many other aspects of the allergic reaction. The allergic reaction may take part in different organs, producing different symptoms, which are sometimes described as different allergic diseases (4).

Sensitization is a precondition for allergy, which includes production of allergen specific IgE antibodies after the first exposure of the allergen (Figure 2, left panel). However, not all sensitized individuals become allergic. Many people are sensitized to one or two allergens without experiencing symptoms when exposed to these allergens under natural conditions. In addition, many individuals who are allergic to one or two allergens may be sensitized to another set of allergens without experiencing symptoms when exposed to these allergens.
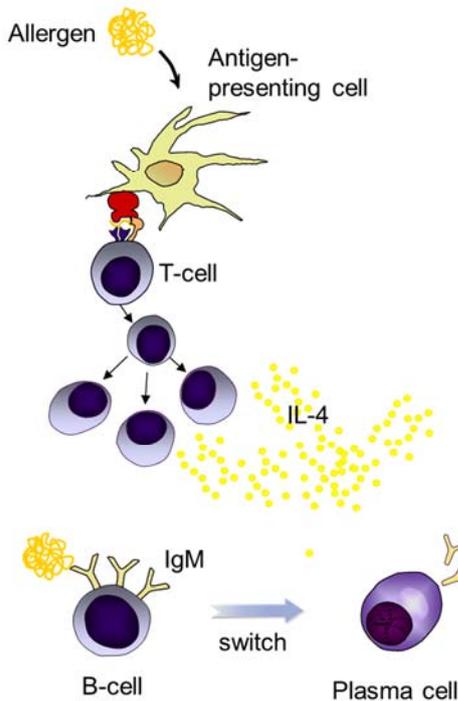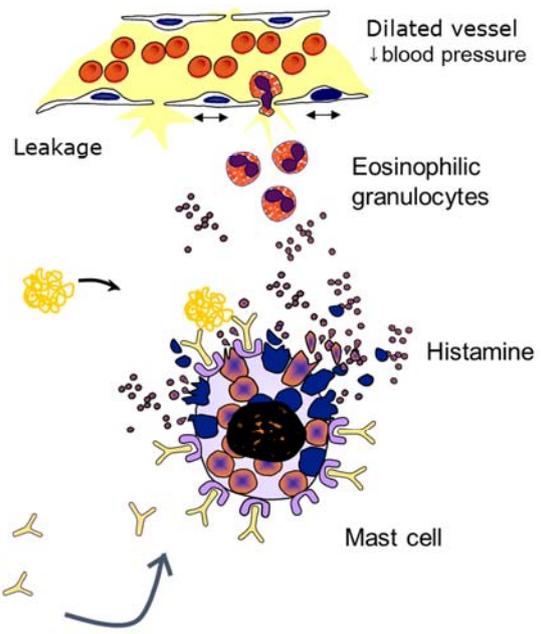
Figure 2. IgE mediated allergy.
Exposure to an antigen (allergen) gives rise to IgE antibody producing plasma cells in a sensitized individual (left panel). Sensitization is a precondition for allergy, although not all sensitized individuals become allergic. An allergic individual shows allergic symptoms after renewed exposure to the same allergen. The symptoms are initially mediated by histamine that is released from mast cells, which have been activated by the cross-linkage of allergen specific IgE antibodies (right panel). Based on illustration in the book *Inflammation* by Mölne and Wold (4).

The underlying defect in IgE mediated allergy

Production of IgE antibodies occurs in the local lymph nodes. After ingestion or inhalation of an allergenic protein, small doses of the protein pass across the mucosa in an intact form. According to "the dual allergen hypothesis" by Gideon Lack, sensitization of the infant may often occur via the skin, which is the best route to induce powerful immune responses (23, 24). Regardless of the route, the allergen that has been taken up is ingested by an antigen presenting cell, also termed dendritic cell. The dendritic cell digests the allergen into peptides and moves, via local lymph vessels, into the nearest draining lymph node. In the lymph node, peptides are presented on the surface of the dendritic cell to naïve T cells that circulate through the lymph node. If a T cell happens to have the right specificity, it will bind to the antigen peptide, become activated and divide (proliferate). Thus, from one activated antigen-specific T cell, perhaps a thousand T cells with identical antigen specificity will be produced (4).

Intact antigen will also be transported to the lymph node in soluble form. B cells recognize their antigen via membrane-bound antibodies on their surface. If a naïve B cell circulating through the lymph node happens to meet its specific antigen there, the antigen will bind to its surface and initiate activation of the B cell. However, the B cell requires help from an activated T cell to become a plasma cell that produces specific IgE antibodies against the allergen. Th2 cells, producing IL-4,

IL-5, IL-9 and IL-13, are crucial for this help. The activated Th2 cell needs to be present in the same lymph node and needs to have been activated by the same allergen (see above) (4).

When the B cell has matured into a plasma cell, the plasma cell stays in the lymph node, producing large amounts of IgE antibodies specific for the allergen. These antibodies exit the lymph node via the lymph and, as the lymph vessels empty into the blood circulation, the antibodies will occur in the blood stream, from which they exit to bind to the surface of mast cells located around blood vessels (see above).

It is far from clear what makes certain individuals produce IgE antibodies to common dietary and inhalant proteins that have always been present in our environment. In 1911, Wells sensitized guinea pigs by injections of miniscule amounts of different plant proteins and elicited an anaphylactic reaction in the animals by injecting the same protein some weeks later (25). One group of guinea pigs did not react with anaphylaxis to the protein from maize (corn) that he had injected into them. Wells researched the history of this group of guinea pigs and found that they had been fed corn by their breeder. He, thus, discovered the oral tolerance phenomenon, i.e. that a healthy animal becomes actively tolerant to a protein that is ingested, meaning that the animal cannot be sensitized at a later time-point (and, hence, not become allergic) to the same protein.

Oral tolerance has been studied for 100 years, but the mechanism is still somewhat obscure. Chase showed in the 1940s that experimental animals for contact dermatitis became unreactive if they were fed the allergen beforehand (contact allergy is an allergic reaction to small fat-soluble chemicals that diffuse through the skin, typically nickel and chromium) (26).

In 1994 Steffen Husby showed that also human beings could become tolerant by being fed an antigen (27). He gave the antigen KLH (a protein that is not present in our diet or surroundings) to volunteers. When he later gave these people an injection of KLH, he noted that those fed KLH were tolerant to the protein, i.e. their T cells were not activated upon immunization. Today, it is thought that oral tolerance involves presentation on specialized dendritic cells in lymph nodes draining the gut in a manner that leads to activation of T cells of the regulatory phenotype. These antigen-specific regulatory T cells will suppress the activation of other T cells that are specific to the same antigen.

It was shown already in 1911 by Wells (25) and was also shown in 1989 by Lamont (28) that oral tolerance requires ingestion of a substantial amount of the protein in question, while smaller doses are more prone to elicit sensitization. Further, exposure to an antigen via the skin is likely to result in sensitization, while exposure via the gut is more likely to result in oral tolerance (23, 24).

ALLERGY DEVELOPMENT IN FARM CHILDREN

As early as 1873, Blackely reported a lower prevalence of hay fever in farming families (9). However, not until the later part of the 20th century was the association between farming and atopy was evaluated in an emerging number of studies (29-32), in parallel with the increased prevalence of allergy in countries with a Western lifestyle (2). Growing up on traditional family farms has thereafter repeatedly been observed to confer strong protection from allergy development (33), and the prevalence of allergy is markedly low in farmers and their children, as reviewed by von Mutius and Vercelli (10). Many of the studies, in which the farm exposure primarily was investigated in childhood, have included dairy farmers (29, 34, 35). However, other animals, such as horses, pigs and poultry were often kept as well. Contact with livestock has been pointed out as one of the major factors that contribute to the farm protection (34); the more frequent the contact (12, 13, 36) and the higher the number of different types of animals (37), the stronger the protection.

Keeping animal feed (34, 36) and the consumption of unpasteurized milk (38) are two other components identified to account for part of the protection.

Being exposed to protective factors from the farming environment very early in life has been shown to be of great importance (36, 37, 39). In a study with a cross-sectional setting of six to 13-year-old children, Reidler and coworkers retrospectively examined early farming exposures (39). They found significant differences in allergy prevalence among those children that had been exposed to stables and consumed farm milk before the age of one year, compared to children with the same exposures, but between the ages of one and five years. The lowest frequencies of allergy were found in children with continous long-term exposure to stables up to the age of five years (39). Douwes and coworkers investigated not only early exposure of the child, but the prenatal exposure through pregnant mothers and allergy prevalence in five to 17-year-old children (36). Children of mothers who were exposed to allergy protective 'farm factors' (farm animals and feed) during pregnancy had a lower prevalence of allergy than children who were only themselves exposed to these farm factors during their first two years, or currently at the age of five to 11 years. The strongest effect was found for the combination of maternal exposure and current exposure (36). Ege and coworkers also found indications of the in utero time to be of importance, finding a lower prevalence of atopic sensitization in children whose first contact with farm animals was in utero, through their mothers, compared to children whose first contact was in their first year of life, who in turn had lower prevalence than children whose first contact with farm animals was after one year of age (37).

Despite strong indications of the importance of exposure to protective factors early in life, most studies have been retrospective in design, conducted in subjects from the age of four years and upwards, through to adulthood. Only a limited number of studies have included children below one year of age (40, 41). Merchant and coworkers included children aged 0 to17 years in their cohort, although with a mean age of nine to ten years (40). However, as other retrospective studies that measure the outcome prior to the exposure, allergy prevalence was evaluated rather than allergy development. The researchers found a lower prevalence of allergies in children raised on a farm compared to children not raised on farms. The significance became a tendency when instead children currently living on a farm were compared with children not currently living on a farm (80% of those currently living on a farm and 12% of those not currently living on a farm were born on a farm). No significant differences were found between the groups regarding asthma (40). In contrast to cross-sectional studies, Midodzi and coworkers evaluated the cumulative *incidence* of parental reported doctor-diagnosed asthma two years after the inclusion of asthma-free children aged 0 to 11 years (17% aged 0 to one year) (41). Children from farming families had a significantly reduced risk of developing asthma compared to children from rural non-farming families, while the incidence of asthma was less pronounced and only insignificantly lower in children from rural non-farming families compared to urban children. The protective 'farm effect' was lost when only children aged six to 11 years were analyzed, which might be a result of fewer children being exposed to the farm environment in their early life; unfortunately, no history of the children's farm residence was reported (41).

DIETARY PATTERN OF FARMERS IN EUROPE

Studies of dietary habits of farmers in the Western world are scarce, and none exist regarding the dietary habits of very young children living on farms, to the author's knowledge. Studies of the diet of farmers all point in the same direction, i.e. a higher intake of full-fat dairy and/or farm produced foods in farming families (11-14, 42). In 1992, Lande and Almås aimed to map the dietary habits of Norwegian farmers (42). In total, approximately 1 866 farming men and women were included in the study; no comparison was made with non-farming subjects. Thirty-eight percent of the

farmers used butter, while 25% consumed margarine on breads, and the majority (55%) drank whole milk. The farmers consumed approximately the same amounts of homemade bread as shop bought, and the majority consumed semi wholegrain bread. Also, the majority consumed fresh fruit two to three or four to six times a week, and eight to nine meals containing meat and two to three meals containing fish each week (42). Almost ten years later (2000), Ehrenstein and coworkers found a higher intake of whole milk, but not skim milk, in farmers' (n = 1 181) compared to non-farmers' (n = 8 466) children of five to seven years of age, living in rural areas of Germany (13). In 2003, a study including 366 farm and 344 non-farm children aged six to 13 years from mainly rural areas in eastern Finland was conducted by Remes and coworkers (12). The use of butter spreads on bread was higher in the farmers' children compared to non-farmers' children (52% vs. 32%), who instead consumed more margarines (67% vs. 44%); a similar trend was found for cooking. The farm children had a higher intake of farm milk or whole milk than did the control children (37% vs. 4%), who consumed more skim milk (32% vs. 18%). The control children in this study, also consumed fish of any kind 'at least once a week' more often than the farm children (33% vs. 25%), although no difference in the intake of oily fish was found (5% controls vs. 7% in farm children) nor in the intake of eggs, yoghurt, meat or whole grain products (12).

The 'Prevention of Allergy-Risk factors for Sensitization In children related to Farming and Antroposophic Lifestyle' (PARSIFAL) study was initiated in the year 2000 and included approximately 2 800 farm children and 5 440 control children aged five to 13 years from rural areas in Austria, Germany, the Netherlands, Sweden and Switzerland (33). A larger number of children from farming families had a regular consumption of the following foods, produced at the farm: milk (67% vs. 20%), yoghurt (39% vs. 17%), butter (39% vs. 14%), eggs (69% vs. 39%) and vegetables (84% vs. 56%) (14). The shop-purchased foods milk, butter, eggs and meat were also consumed regularly by a higher number of farmers' children, although the difference was less pronounced as compared to home produced foods. In contrast, a higher number of control children consumed margarine and olive oil regularly, while no differences were found in the consumption of fruits and whole grains (11).

TIMING OF INTRODUCTION OF COMPLEMENTARY FOODS AND ALLERGY DEVELOPMENT

An introduction of complementary foods and a high diversity in the diet of the weaning infant before the age of four months was proposed to increase the risk of atopic diseases in a number of early epidemiological studies (43, 44). In 2000 a position statement was released by the American Academy of Pediatrics regarding infant feeding practices and hypoallergenic formulas (45). With reservation that conclusive studies were not yet available to permit definitive recommendations, the authors recommended that breastfeeding mothers of high-risk children should eliminate peanuts and nuts, and consider eliminating eggs, cow's milk and fish from their diet during lactation. Exclusive breastfeeding was recommended to the age of six months, before which solid foods were not to be introduced; further, dairy products were recommended to be delayed until one year, eggs until two years, and peanuts, nuts, and fish until three years of age (45). Based on a growing number of subsequently published studies showing no protection when introduction of potentially allergenic foods were delayed, the American Academy of Pediatrics released an updated position statement in 2008 (46). The authors summarize that, at the present time, no clear evidence exist that either maternal avoidance during pregnancy or delaying the introduction of potentially allergenic foods until after the age of four to six months is preventive for allergy development. However, complementary foods were still not recommended before the age of four to six months (46). Even more strict recommendations are given by the Swedish National Food Agency, advising

Table 1. Inverse associations between timing of fish introduction and development of allergy and sensitization.

Study	Subjects	Study design	Reverse causation	Clinical outcome	Findings
Lumia et al. 2015 (58)	n = 182 cases n = 728 matched controls DIPP study	Birth cohort; case-control study. Data on timing of food introduction collected <2 yr by questionnaires.	Adjustment for early cow's milk allergy.	Parental-reported atopic asthma and non-atopic asthma at 5 yr; clinical measurements of IgE.	Higher fish intake <4 yr decreased risk of all asthma; the significance disappeared after adjustment for age at fish introduction.
Nwaru et al. 2013 (48)	n = 3 781 DIPP study	Birth cohort Questionnaires at 3, 6 and 12 mo; a form for introduction of new foods sent by mail at 2 mo, kept until 2 yr and checked every clinic visit.	Reverse causality: Stratification by eczema at 6 mo and parental allergic history, if interaction with the food variables.	Atopic sensitization (0.35 kU/l) to wheat, fish, egg, milk, timothy grass, birch, cat and house dust mites, at 5 yr.	Introducing fish <6 mo decreased the risk of sensitization to all allergens; stronger effect in high-risk children.
Nwaru et al. 2013 (47)	n = 3 781 DIPP study	Birth cohort Questionnaires at 3, 6 and 12 mo; a form for introduction of new foods sent by mail at 2 mo, kept until 2 yr and checked every clinic visit.	Reverse causality: No interactions between atopic eczema at 6 mo and parental heredity and introduction of any foods.	Allergic rhinitis and doctor-diagnosed asthma and atopic eczema at 5 yr; all parental-reported; clinical measurements of IgE.	Introducing fish ≤9 mo decreased the risk of allergic rhinitis and atopic sensitization.
Kiefte-de Jong et al. 2012 (56)	n = 7 210	Birth cohort Questionnaires at 12 and 14 mo.	Sensitivity analyses of asthmalike symptoms at 12 and 24 mo.	Parental-reported asthmalike symptoms (wheezing) at 3 and 4 yr.	Introducing fish 6-12 mo decreased the risk of asthma at 4 yr, compared to introducing <6 or >12 mo.
Goksör et al. 2011 (60)	n = 4 496 'Infants of Western Sweden'	Birth cohort Questionnaires at 12 mo.	Atopic heredity, eczema and doctor-diagnozed food allergy controlled for in multivariate analysis.	Parental reported recurrent wheeze at 4.5 yr.	Introducing fish <9 mo decreased the risk of wheeze.
Hesselmar et al. 2010 (55)	n = 184 5/6 children had at least one parent with a history of allergy	Birth cohort	Reversed causation: Eczema at 6 mo; wheeze at 6 mo; reactions to cow's milk at 6 mo; heredity (excl. food allergy).	Clinical diagnoses at 18 mo: Eczema, asthma, food allergy and sensitization.	The earlier the introduction of fish, the lower the frequency of eczema; also asthma frequency tended to be lower.

Study	n / population	Design	Analysis	Outcome	Conclusion
Alm et al. 2009 (54)	n = 4 921 'Infants of Western Sweden'	Cohort Questionnaires at 6 and 12 mo.	Log regression including maternal heredity and cow's milk allergy at 1 yr?	Parental reported previous/current eczema at 1 yr.	Introducing fish <9 mo decreased the risk of eczema.
Kull et al. 2006 (57)	n = 4 089	Birth cohort Questionnaire at 12 mo.	Exclusion of chidren with eczema or wheeze ≤1 yr. Also parental heredity was considered. Effects weakened, but remained when frequency of fish consumption at 1 yr and allergy was evaluated. Not clear wheather relationships between fish introduction and allergy were evaluated.		Introducing fish between 3-8 mo decreased the risk of asthma, eczema and allergic rhinitis, compared to ≤9 mo.
Nafstad et al. 2003 (59)	n = 2 531	Birth cohort Questionnaire at 12 mo.	Stratification by parental atopy, atopic eczema 0-6 mo, an episode of lower respiratory tract infection ≤1 yr.	Parental-reported doctor-diagnosed asthma and allergic rhinitis at 4 yr. Secondary outcome: current atopic dermatitis.	Introducing fish ≤1 yr decreased the risk of allergic rhinitis and a weaker decreased risk of asthma and atopic dermatitis.

The studies are sorted by age of the subjects.

exclusive breastfeeding up to the age of six months, although tiny amounts of 'taste sensations' are regarded as tolerable.

In recent years, studies have shown an increased risk of allergy development and atopic sensitization by a delayed introduction of complementary foods (47-50) or a low diversity (48, 51-53) in the weaning diet. In studies where the timing of the first exposure to fish has been evaluated, a postponed introduction has repeatedly been observed to increase the risk of different atopic manifestations (47, 48, 54-60). The studies, in which positive associations between allergy and late fish introduction have been found are compiled in Table 1.

The old recommendations regarding postponing the introduction of potentially allergenic foods clearly modified the introduction practices of complementary foods (61, 62). Despite the new consensus among allergy and pediatric associations regarding postponed introduction not being linked to allergy protection (46), this notion still lingered on among allergists and nutritionists (63). A delayed introduction of complementary foods, and especially allergenic foods, among high-risk infants may therefore result in false negative associations between early introduction and allergy protection. This phenomenon is termed reverse causation, which means that parents to children with early symptoms of allergy or with allergy in the family delay the introduction of potentially allergenic foods as an attempt to decrease the risk of allergy development. As these children are at higher risk of developing allergies, a higher incidence of allergies among those children may be a result of heredity rather than of delayed introduction. Hence, it is important to consider reverse causation when epidemiological studies are carried out in this area.

In addition to reverse causation, observational studies are subject to potential covariance among the exposure of interest and established (or yet unestablished) risk factors and the outcome of interest. Hence, an apparent association between the exposure and outcome of interest may be mediated by a covariate, for example parental allergy, which predisposes the offspring to allergy development. In randomized controlled trials, potential confounders are evenly distributed among the test groups, and the risk for reverse causation is diminished, which enables the evaluation of a causal relationship between exposure and outcome. Until 2015, no studies evaluating the timing of introducing complementary foods and allergy development in an RCT setting were published. The Learning Early about Peanut Allergy (LEAP) study, was the first of its kind, comprising 640 infants between four and 11 months of age at high risk of developing peanut allergy, randomly assigned to either avoid or consume regularly substantial amounts of peanut protein up to the age of five years (64). The early-introduction group had a markedly lower risk of developing peanut allergy at five years of age, compared to the avoidance group, and the results were even more pronounced in those of the exposure group that were sensitized to peanut at baseline. The LEAP study was closely followed by the Enquiring About Tolerance (EAT) study, in which 1 303 three-month-old infants at general risk were randomly assigned to a group of early introduction of peanut, cooked egg, cow's milk, sesame, white fish, and wheat, or to a control group with instructions of current recommendations of exclusive breastfeeding up to six months of age (65). The prevalence of any food allergy was lower in those children whose parents managed to introduce substantial amounts of foods early, in accordance with the protocol, compared to controls. The same was observed for peanut and egg allergy. No adverse effects of early introduction were observed in either the LEAP study or in the EAT study (64, 65).

The importance of early introduction of complementary foods, including potential allergens, is underpinned by a number of studies in which the process of becoming tolerant to a substance via the oral route has been evaluated. Normally, an antigen-specific active immune tolerance is induced by a dietary protein, a course of event termed oral tolerance. This has been shown in experimental animals (66, 67) and in humans (27). Achieving tolerance requires feeding substantial amounts of the protein is required, as shown by animal experiments (25, 28). To the contrary, small doses may

induce sensitization and hence a predisposition to develop allergy (25, 28). In the dual exposure hypothesis, Gideon Lack has proposed that miniscule exposures of potential allergens via the cutaneous or inhalant route, in place of the oral route, may result in sensitization and allergy development rather than tolerance (23, 24).

CONSUMPTION OF DAIRY PRODUCTS AND ALLERGY DEVELOPMENT

Consumption of butter (14, 16, 17, 68-70) and dairy products (15, 53, 71-75) has been associated with a decreased risk of sensitization and allergy. Studies in which inverse associations between butter and milk products have been found are summarized in Table 2. Both Snijders *et al.* (73) and Roduit *et al.* (53) found an increased risk of eczema if introduction of cow's milk and yoghurt, respectively, was delayed to the weaning diet; this was also the case after considering reverse causation. Further, consumption of regular milk was associated with atopy in six studies (15, 16, 70-72, 75). A decreased risk of eczema (70, 71), asthma/wheeze (16, 75), allergic rhinitis (15) and atopic sensitization (72) was observed in the studies, of which two had cross-sectional design (70, 75). In one of the studies, a significant association was found only for organic dairy products, which was still significant after adjustment of both raw and boiled farm milk (71). An inverse association between butter consumption and respiratory allergy was observed in one birth-cohort (16) and two cross-sectional studies (69, 76); the same was observed in a retrospective study for hay-fever (17). In a case-control study of children with atopic diseases and their references, butter intake was also found to be negatively associated with allergy (68). In line with these findings, a high maternal intake of saturated fatty acids during pregnancy was found in a Finnish birth-cohort to be associated with a decreased risk of asthma in the offspring at the age of five years (77). The allergy diagnoses were self- or parental-reported in all studies except for the study by Johansson et al., in which the allergic state was confirmed by analysis of total IgE and specific IgE antibodies in blood samples (69). However, this study was the only study in which potential confounding was not accounted for (69).

In contrast to the above mentioned studies showing a potential protective effect of butter and milk intake, results showing positive associations between atopic diseases and the consumption of these foods have been observed as well (12, 15, 72, 78-81). Even more notable, however, is that the consumption of farm milk, and especially unpasteurized farm milk, has been observed to confer protection against atopic diseases (36, 38, 39, 82) (H). Loss and coworkers showed that intake of raw farm milk was inversely associated with rhinitis and respiratory tract infections in the first five years of life, when compared to ultra heat-treated milk; the same associations were observed for boiled farm milk, although weaker (83). The protective effect of consumption of raw farm milk does not seem to be confined only to those living on a farm, as demonstrated by Waser and coworkers, who observed a lower risk for asthma and allergic rhinitis both in farm resident and non-farm resident children consuming unpasteurized milk (76).

Table 2. Inverse associations between the intake of butter and dairy products and atopy.

Study	Subjects	Study design	Confounder control	Clinical outcome	Findings
Snijders et al. 2008 (73)	n = 2 558 The Netherlands	Birth-cohort Dietary questionnaires at 3, 7, 12 and 24 mo	Exclusion of children with early symptoms of eczema and recurrent wheeze	Parental-reported eczema, atopic dermatitis, recurrent wheeze and atopic sensitization at 2 yr	More delay in cow's milk introduction increased the risk of eczema
Roduit et al. 2012 (53)	n = 2 978 Austria, Finland, France, Germany, Switzerland	Birth-cohort Monthly dietary diaries at 3-12 mo	Exposure occured after onset of disease	Parental-reported doctor-diagnosed atopic dermatitis between 1-4 yr	Introducing yoghurt after the 1 y of age increased the risk of atopic dermatitis
Kummeling et al. 2008 (71)	n = 2 764 0-2 yr The Netherlands	Birth-cohort Dietary questionnaire at 2 yr	Sex, maternal education, infant BMI at 1 yr, parental and sibling history of allergy, older siblings, breastfeeding, day-care attendance, pets, tobacco smoke, vaccinations, antibiotica, vegetarian diet, raw/farm milk intake, maternal exclusion of milk	Parental-reported eczema and wheeze ≤2 yr	Intake of organic dairy products decreased the risk of eczema
Wijga et al. 2003 (16)	n = 2 978 2-3 yr The Netherlands	Birth-cohort FFQ at 2 yr	Sex, birth weight, older siblings, parental asthma, maternal education, breastfeeding ≤8 wk, maternal smoking during pregnancy, smoking in the home at 1 yr of age, region.	Parental-reported doctor-diagnosed asthmaa, parental-reported wheeze at 3 yr	Milk and butter intake decreased the risk of wheeze; full cream milk and butter intake decreased the risk of asthma
Nwaru et al. 2011 (72)	n = 652 0-5 yr Finland	Birth-cohort FFQ, lactation (3 mo postpartum)	Sex, place of birth, duration of gestation, maternal smoking during pregnancy, mode of delivery, parental asthma and allergic rhinitis, atopic eczema at 6 mo, exclusive breastfeeding	Atopic sensitization	Maternal milk intake decreased the risk of sensitization at 5 y
Suárez-Varela et al. 2010 (70)	n = 13 153 6-7 yr Spain	Cross-sectional Dietary questionnaire	Gender, obesity, tobacco smoke at 1 yr, younger and older siblings, exercise	Parental-reported atopic dermatitis	Milk intake decreased the risk of atopic dermatitis
Farchi et al. 2003 (15)	n = 4 104 6-7 yr Italy	Prospective Dietary	Sex, study area, paternal education, household crowding, maternal and paternal smoking,	Parental-reported wheezing and allergic rhinitis 1 year	Milk intake decreased the risk of allergic rhinitis

12

				questionnaire at 6-7 yr	dampness/mold in the child's room, paternal asthma	after dietary assessment (7-8 yr)
Waser et al. 2007 (14)	n = 2 823 farm children, 5 440 farm reference children 5-13 yr Austria, Ghermany, the Netherlands, Sweden, Switzerland	Cross-sectional Dietary questionnaire	Study groups (farm, antroposophic, reference groups), country, sex, age, mothers' or fathers' asthma or hay-fever, parents' education, maternal smoking during pregnancy, current smoking at home, older siblings, exclusive breastfeeding >4 mo, BMI, food avoidance due to familial asthma/allergy	Parental-reported doctor-diagnosed asthma, parental-reported wheeze, hay-fever and atopic eczema; validated in a subsample - no difference between groups	Intake of farm and shop purchased butter vs. margarine intake decreased the risk of asthma and wheeze
Von Mutius et al. 1998 (17)	n = 2 334 9-11 yr Germany	Retrospective	Family history of atopic diseases, socioeconomic status, number of siblings, use of wood or coal for heating, pet ownership	Parental-reported doctor-diagnosed hay-fever, atopic dermatitis and asthma	Increased butter intake decreased the risk of hay-fever
Dunder et al. 2001 (68)	n = 154 pairs, atopic and non-atopic, matched for age (= 10 yr), sex and residence area Finland	Case-control, restrospective 48 h dietary recalls	Maternal education	Parental-reported doctor-diagnosed atopic dermatitis, asthma, and allergic rhinitis	Butter intake decreased the risk for atopic diseases
Johansson et al. 2011 (69)	n = 7 respiratory allergy, 16 atopic eczema and respiratory allergy, 22 healthy 30-32 yr, women Sweden	Cross-sectional FFQ, semi-quantitative	No adjustment for potential covariates	Self-reported allergy diagnoses; allergic state confirmed by blood tests of total IgE and specific IgE antibodies	Women with respiratory allergy consumed less butter than healthy women
Woods et al. 2003 (75)	n = 1 601 = 35 yr Australia	Cross-sectional FFQ, semi-quantitative	Age, sex, smoking, BMI, region of birth, family history of asthma	Self-reported doctor-diagnosed asthma	Whole milk intake decreased the risk of asthma

The studies are sorted by age of the subjects. [a]Should be interpreted as asthma-like symptoms due to the young age. FFQ = Food frequency questionnaire.

Table 3. Positive associations between the intake of margarine and atopy.

Study	Subjects	Study design	Confounder control	Clinical outcome	Findings
Sausenthaler et al. 2007 (18)	n = 2 641 0-2 yr Germany	Birth cohort Semi-quantitative FFQ	Study area, sex, maternal age at delivery, maternal smoking during pregnancy, level of paternal education, exclusively breastfed ≤4 mo, parental history of atopic diseases, number of older siblings, birth weight, season of birth	Parental-reported doctor-diagnosed eczema; sensitization measurements ≤2 yr	Maternal intake of margarine and vegetable oils during pregnancy increased the risk of eczema
Lumia et al. 2012 (79)	n = 1 798 0-5 y DIPP study Finland	Birth-cohort Semi-quantitative FFQ	Sex, area of birth, duration of gestation, maternal age, maternal vocational education, maternal smoking during pregnancy, number of previous deliveries, maternal asthma or allergic rhinitis, paternal asthma or allergic rhinitis, atopic eczema by the age of 6 mo, birth weight, mode of delivery, duration of breastfeeding, pets at home, farming contacts to cow shed during 1 yr	Parental-reported doctor-diagnosed asthma at 5 yr	Maternal margarine intake during lactation decreased weakly the risk of asthma
Sausenthaler et al. 2006 (19)	n = 2 582 2 yr Germany	Cross-sectional Semi-quantitative FFQ	Study area, sex, maternal age at delivery, maternal smoking during pregnancy, level of paternal education, exclusively breastfed ≤4 mo, parental history of atopic diseases, keeping a cat, keeping a dog	Parental-reported doctor-diagnosed eczema; sensitization measurements ≤2 yr	Margarine intake decreased the risk of eczema and sensitization; higher effect in children with parental atopy and in boys
Farchi et al. 2003 (15)	n = 4 104 6-7 yr Italy	Prospective Dietary questionnaire at 6-7 yr	Sex, study area, paternal education, household crowding, maternal and paternal smoking, dampness/mold in the child's room, paternal asthma	Parental-reported wheezing and allergic rhinitis 1 year after dietary assessment (7-8 yr)	Margarine intake increased the risk of wheeze
Von Mutius et al. 1998 (17)	n = 2 334 9-11 yr Germany	Retrospective Questionnaire	Family history of atopic diseases, socioeconomic status, number of siblings, use of wood or coal for heating, pet ownership	Parental-reported doctor-diagnosed hay-fever, atopic dermatitis and asthma	Increased margarine intake was associated with a higher frequency of hay-fever
Dunder et al. 2001 (68)	n = 154 pairs, atopic and non-atopic, matched for age (= 10 yr), sex and residence area Finland	Cross-sectional 48 h dietary recalls	Maternal education	Parental-reported doctor-diagnosed atopic dermatitis, asthma, and allergic rhinitis	Margarine intake was higher in the group of atopic children
Waser et al. 2007 (14)	n = 2 823 farm children, 5 440 farm reference children 5-13 yr	Cross-sectional Dietary questionnaire	Study groups (farm, antroposophic, reference groups), country, sex, age, mothers' or fathers' asthma or hay-fever, parents' education, maternal smoking during pregnancy, current smoking at home, older siblings,	Parental-reported doctor-diagnosed asthma, parental-reported wheeze, hay-fever and atopic eczema; validated in a	Intake of margarine vs. butter increased the risk of asthma and wheeze

Study	Country / n / age	Study design / Questionnaire	Covariates	Outcome measures	Result
Bolte et al. 2001 (85)	Austria, Ghermany, the Netherlands, Sweden, Switzerland; n = 2 348, 5-14 yr, Germany	Cross-sectional; Dietary questionnaire	exclusive breastfeeding >4 mo, BMI, food avoidance due to familial asthma/allergy; Sex, age, place of residence, parental atopy, presence of siblings, BMI	subsample - no difference between groups; Parental-reported allergic rhinitis, and doctor-diagnosed hay-fever and asthma; sensitization measurements	Margarine vs butter intake increased the risk of rhinitis and sensitization in boys only
Chatzi et al. 2007 (87)	n = 690, 7-18 yr, Greece	Cross-sectional; FFQ	Age, sex, BMI, parental asthma, number of older siblings	Parental-reported allergic rhinitis, wheeze; sensitization measurements	Margarine intake increased the risk of wheeze and allergic rhinitis
Akcay et al. 2014 (84)	n = 9 991, 13-14 yr, Istanbul	Cross-sectional; Dietary questionnaire	Sex, heredity, number of siblings, place of recidence, parental educational level, domestic animals, parental smoking	Parental-reported doctor-diagnosed asthma	Margarine intake increased the risk of asthma
Takaoka et al. 2008 (81)	n = 153 = 21 yr, Japan	Cross-sectional	Age, current smoking, heredity	Self-reported doctor-diagnosed asthma, "asthma symptom score" based on 8 asthma related questions; self-reported allergy to cat, dog, or pollen	Margarine intake was associated with an asthma symptom score
Calvani et al. 2006 (78)	n = 998 children (295 allgergic mothers, 693 non-allergic mothers) Mothers: = 36 yr, Children: = 5 yr, Italy	Cohort; Mothers: cross-sectional; Children: retrospective	Mothers: smoking, education, occupation; Children: e.g. age, gender, number of older siblings, maternal education and occupation	Mothers: self-reported asthma, hay-fever, atopic eczema; Children: atopic sensitization at 5 yr (measurements)	Higher margarine intake during pregnancy in mothers with a history of hay-fever and eczema
Bolte et al. 2005 (86)	n = 7 124, 18-79 yr, Germany	Cross-sectional	BMI, obesity, age, gender, region (East/West), socioeconomic status, smoking	Self-reported asthma, hay-fever, atopic dermatitis	Intake of margarine, foremost low-fat, increased the risk of asthma in young adults (18-29 yr)
Nagel et al. 2005 (88)	n = 105 cases, 420 controls; matched by sex and age, 35-65 yr, Germany	Prospective, case-control; FFQ	Sports, age, fat energy intake, nonfat energy intake, BMI, smoking, gender, educational level	Self-reported doctor-diagnosed asthma, assessed 2 yr after dietary assessment	Margarine intake increased the risk of asthma

15

The studies are sorted by age of the subjects. BMI = Body mass index; FFQ = Food frequency questionnaire. Foods as potential covariates included in the logistic regression models are not displayed. Dietary questionnaire means no specific questionnaire, such as an FFQ, for only foods.

CONSUMPTION OF MARGARINES AND ALLERGY DEVELOPMENT

An increased consumption of margarine and vegetable oils was observed to parallel the increase in atopic manifestations in the late 20th century (20). Consequently, Black and Sharpe hypothesized that margarine and n-6 PUFAs increased the risk of developing allergy (20). Margarine intake has been found in several studies to be positively associated with an increased risk of allergy or atopic sensitization (14, 15, 17-19, 68, 78, 79, 81, 84-89); these studies are summarized in Table 3. In pregnant women, a high margarine intake was associated with eczema in their two-year-old offspring (18); during lactation, maternal margarine intake weakly increased the risk for asthma in the children at five years of age (79). In a cross-sectional study, margarine intake in two-year-old children was associated with a lower risk of having eczema and sensitization (89), and margarine consumption has later in childhood/adolescence been shown to be associated with an increased risk of asthma and/or wheeze (14, 15, 84, 87), hay-fever (17) and allergic rhinitis (85). Also in adulthood, margarine intake has been associated with eczema and hay fever (78), although foremost with asthma (81, 86, 88).

Apart from the analyses of maternal diet during pregnancy and lactation (18, 79), most studies have been of a cross-sectional character (14, 19, 68, 81, 84-87). The birth-cohort by Calvani et al. (78) was also of cross-sectional character regarding the association between atopy and margarine intake, since this concerned the mothers, for whom both exposure and reports of outcomes were assessed at the same time. Another study was retrospective in its design (17), while two other were prospective, spanning over one (15) and two (88) years. Confounding was considered in all studies, although, in the study by Dunder et al. (68), maternal education was the only potential covariate accounted for. Of all diagnoses, asthma appears to be most consistently associated with margarine intake (14, 15, 79, 81, 84, 86-88); however, the atopic character of all of the asthma diagnoses is unclear.

In contrast to the abovementioned studies, in which positive associations were observed between margarine intake and allergy, no association (16, 90, 91) or even an inverse association (70, 72), were observed by others.

CONSUMPTION OF FISH AND ALLERGY DEVELOPMENT

Consumption of fish has repeatedly been associated with a decreased risk of allergy development in children. Associations have been observed for fish that is introduced early to the child's diet (47, 48, 54-59, 92-94) as well as for the mother's intake during pregnancy (18, 21, 78, 91, 95-97) and for fish in the regular diet of toddlers (22, 57) and children (90, 98-101). Further, the intake of fish and seafood among 21-year-old Japanese students was associated in one study with fewer respiratory infections and pollen allergy, respectively (81). Studies in which inverse associations between fish intake and allergy have been found are summarized in Table 4. Six of the studies were prospective (18, 22, 57, 91, 95, 96), while two were retrospective (78, 97) and five had cross-sectional designs (81, 90, 98-101). One was a population-based controlled intervention study in which asthma at two years of age was significantly lower in the intervention group; the intervention group had an increased intake of oily fish and fish oil both in the diet of the children as well as their mothers during pregnancy and lactation (21). However, the character of asthma was not clearly specified (21), as in most of the other studies where asthma or asthma-like symptoms have been evaluated (22, 57, 81, 90, 91, 97, 99-101); only in two studies were wheeze or asthma defined as atopic (96, 98). The allergy outcomes were also in all studies reported by the parents (or the subjects themselves (81)); half of the studies included parental/self-reports of doctor diagnoses for some

16

of the allergic phenotypes (18, 21, 22, 81, 91, 95, 97). In one study, the parents' reports of atopic asthma and wheeze were strengthened by measurements of atopic sensitization (98).

In almost half of the studies in which an inverse association between fish and allergy was found (Table 4), the mothers' intake of fish during pregnancy was inversely related to allergy development in the offspring (18, 21, 78, 91, 95-97), which points to the importance of early exposure to immune stimulating factors in the etiology of atopic diseases. An exception was the study by Øien and coworkers, in where the intake of fish in one-year-old children, but not in the pregnant mothers, was associated with a lower risk of developing eczema at two years of age (22). However, the mothers' diet was assessed retrospectively when the child was one year (22), which may increase the risk of recall bias. The opposite was shown by Willers and coworkers, who reported on associations between maternal fish intake during pregnancy and a decreased risk of eczema in the children up to the age of two years; no associations were found between eczema and the children's fish intake, assessed at five years of age (91). Moreover, both Goksör (60) and Kiefte-de Jong (56) and their coworkers found that introduction of fish before the age of nine months and between six to 12 months, respectively, were inversely associated with wheeze at four years; no effect was found for consumption of fish at 12 (60) and 14 (56) months of age. However, the consumption of fish at 12 months in the study by Goksör and coworkers (60) became borderline significantly associated with a decreased risk of atopic asthma at eight years of age (92).

Although nutritional exposure already *in utero* seems to be of great importance, fish intake in childhood has also been inversely associated with allergy in several studies (22, 57, 90, 98-100) (Hodge 1996). However, the children's diet may well be a proxy for the mothers' diet, and vice versa. Romieu and coworkers addressed this question by considering the child's fish intake at four years of age as a potential confounder, while assessing the relation between the mother's fish intake during pregnancy and atopic outcomes in the children; an inverse association between maternal fish intake and eczema at one year of age as well as atopic wheeze at six years of age was still observed (96).

POTENTIALLY ALLERGY PROTECTIVE COMPONENTS IN FISH

Long-chain omega-3 polyunsaturated fatty acids

The inverse association observed between fish intake and allergy has been ascribed to the content of long-chain n-3 PUFAs in a number of studies (22, 97, 102, 103). Maternal consumption of oily fish during pregnancy was associated with a decreased risk of asthma in the children at five years of age, whereas no effect was found for the intake of lean fish, as reported by Salam and coworkers (97). Øien and coworkers showed that fish intake at one year of age decreased the risk of eczema at two years of age; the effect was stronger for oily fish than for lean fish, although no effect was observed for cod liver oil (22). Both Hoppy (102) and Miyake (103) and their coworkers found inverse associations between long-chain n-3 PUFA exposure in infancy and subsequent allergy development. In the study by Hoppy and coworkers, higher proportions of EPA in breast milk were negatively associated with atopic dermatitis up to the age of one year (102). No effect was observed for maternal fish intake during pregnancy (102). In the study by Miyake and coworkers, maternal intake of the n-3 PUFAs DHA and alpha-linolenic acid during pregnancy was inversely associated with wheeze but not eczema at 16-24 months of age, while no associations were found for fish intake (103). In addition, the inverse association observed in several studies between consumption of unpasteurized farm milk and allergy (36, 38, 39, 82), has partly been ascribed to the content of n-3 PUFAs in one study (104).

Table 4. Inverse associations between fish intake and allergy.

Study	Subjects	Study design	Confounder control	Clinical outcome	Findings
Jedrychowski et al. 2011 (95)	n = 469 0-1 yr Poland, USA	Birth cohort FFQs twice during pregnancy	Maternal characteristics (age, education, atopy), duration of exclusive breastfeeding, presence of older siblings and damp/moldy house	Parental-reported doctor-diagnosed eczema ≤1 yr (reported by interviews 3, 6, 9 and 12 mo postpartum)	Maternal fish intake during pregnancy decreased the risk of eczema
Dotterud et al. 2013 (21)	n = 1 374 (intervention), 4 780 (control) 0-2 yr Norway	Controlled intervention Increased oily fish and n-3 PUFA intake in both mothers and children; reduction in parental smoking Semi-quantitative FFQ	Maternal age at delivery, birthweight, siblings, born pre-term, single maternity, breastfeeding, maternal atopy, paternal atopy, double parental atopy, having dogs, having cats, homeowner	Parental-reported doctor-diagnosed asthma, parental-reported wheeze and atopic dermatitis at 2 yr	Intake of n-3 PUFAs and oily fish decreased the risk of asthma; stronger effect on girls
Sausenthaler et al. 2007 (18)	n = 2 641 0-2 yr Germany	Birth cohort Semi-quantitative FFQ	Study area, sex, maternal age at delivery, maternal smoking during pregnancy, level of paternal education, exclusively breastfed ≤4 mo, parental history of atopic diseases, number of older siblings, birth weight, season of birth	Parental-reported doctor-diagnosed eczema; sensitization measurements ≤2 yr	Maternal intake of fish during pregnancy decreased the risk of eczema
Willers et al. 2007 (91)	n = 1 212 0-5 yr The Netherlands	Birth cohort Mothers: FFQ during pregnancy Children: FFQ at 5 yr	Maternal age, paternal social class, maternal age of leaving full time education, maternal smoking during pregnancy, maternal asthma, maternal atopy, child's sex, child's birth weight, presence of older siblings, breast feeding, and smoking in the child's home at 5 yr	Parental-reported doctor-diagnosed asthma, eczema and hay-fever, and measurements of sensitization at 5 yr	Maternal fish intake during pregnancy decreased the risk of eczema; no association for the children's intake
Salam et al. 2005 (97)	n = 279 cases, 412 controls (0-)5 yr USA	Nested case-control study Assessment of maternal fish intake during pregnancy by telephone interview, retrospectively	Maternal smoking during pregnancy, time of asthma onset, paternal history of asthma, second-hand tobacco exposure, yearly family income, gestational age, maternal age, maternal education, and race	Parental-reported doctor-diagnosed asthma at 5 yr	Maternal oily fish intake during pregnancy decreased the risk of asthma, in children to mothers with a history of asthma but not in children to healthy mothers; no effect of lean or canned fish
Calvani et al. 2006 (78)	n = 998 children (295 allergic mothers, 693 non-allergic mothers) (0-)5 yr Mothers: mean =	Retrospective Questionnaire	Mothers: smoking, education, occupation Children: e.g. age, gender, number of older siblings, maternal education and occupation	Atopic sensitization at 5 yr (measurements)	Maternal fish intake during pregnancy decreased the risk of sensitization

Study	Population	Design/Method	Confounders adjusted for	Outcome assessment	Results
	36 yr Children: mean = 5 yr Italy				
Romieu et al. 2007 (96)	n = 462 0–6 yr Spain	Birth cohort FFQ	Gender, maternal age, maternal and paternal atopy, maternal and paternal asthma, maternal and paternal social class, maternal smoking during pregnancy, maternal BMI before pregnancy (as proxy for caloric intake), gender, gestational age, birth weight, parity, breastfeeding, ownership of pets, BMI at age 6.5 yr, dichlorodiphenyldichloroethylene in cord blood, and the child's fish consumption at age 4 yr (using the same scoring system that for the mother's fish intake)	Parental-reported at 1 yr, eczema, and atopic wheeze at 6 yr; measurements of sensitization at 4 and 6 yr	Maternal fish intake during pregnancy decrease the risk of eczema at 1 yr and of wheeze and sensitization at 6 yr; effect on wheeze only observed in non-breastfed children
Øien et al. 2010 (22)	n = 3 086 1–2 yr Norway	Prospective Mothers: Diet during pregnancy assessed retrospectively Children: semi-quantitative FFQ; both assessments at 1 yr	Eczema ≤1 yr, gender, familial atopy, parental smoking 1 year after delivery, children's consumption of cod liver oil, parental homeowner status as a proxy for social class during the first year of life, and exclusive breastfeeding	Parental-reported doctor-diagnosed asthma and parental-reported eczema ≤2 yr	Fish intake decreased the risk of eczema; effects were stronger for oily fish than for lean fish; no effect for cod liver oil; no effect from maternal intake of fish or cod liver oil
Kull et al. 2006 (57)	n = 3 614 1–4 yr Sweden	Birth cohort Dietary questionnaire at 1 yr	Parental allergic disease, maternalage, maternal smoking, breastfeeding, eczema ≤1 yr, eczema/wheeze ≤1 yr	Parental reported asthma, atopic eczema, allergic rhinitis, multiple allergic diseases (at least 2 of the above deseases), and measurements of atopic sensitization at 4 yr	Fish intake decreased the risk of eczema, allergic rhinitis, any allergic disease and sensitization
Chatzi et al. 2007 (90)	n = 460 6.5 yr Spain	Cross-sectional FFQ	Gender, maternal and parental asthma, maternal and paternal atopy, maternal smoking, BMI at age 6.5 yr, maternal and paternal education and social class, breastfeeding, fish intake during pregnancy, and number of siblings at age 6.5 yr	Parental-reported wheeze, measurements of sensitization (n = 412)	Fish intake decreased the risk of sensitization
Antova et al. 2003 (99)	n = 20 271 7–11 yr Bulgaria, Czech Republic, Hungary,	Cross-sectional Dietary questionnaire	Age, sex, area, presence of pets, presence of indoor moisture, use of gas oven for heating, additional unvented gas heating, number of smokers in household, mother's education, father's occupation, parents' allergy, respondent (person that filled out the questionnaire), overcrowding.	Parental-reported wheeze	Fish intake decreased the risk of wheeze

Study	Country, n, age	Design (questionnaire)	Covariates	Outcome	Results
Hodge et al. 1996 (101)	Poland, Romania and Slovakia; n = 468; 8-11 yr; Australia	Cross-sectional (outcome measured before exposure) FFQ	Sex, ethnicity, country of birth, atopy, respiratory infection in the first two years of life and a parental history of asthma or smoking	Parental reported asthma, measurements of atopic sensitization; assessed 6 mo before dietary assessment	Intake of oily fish decreased the risk of asthma; stronger effect on girls
Arvaniti et al. 2011 (100)	n = 700; 10-12 yr; Greece	Cross-sectional Dietary questionnaire	Age, sex, BMI, physical activity, energy intake	Parental-reported asthma	Fish intake decreased the risk of asthma symptoms
Tabak et al. 2006 (98)	n = 598; 8-13 yr; The Netherlands	Cross-sectional Semi-quantitative FFQ	Maternal educational level, foreign descent, total energy intake, age, sex, number of siblings, ever asthma in siblings, ever asthma in parents, smoking of the mother during pregnancy, being breastfed, passive smoking, pets at home, and BMI	Parental-reported atopic asthma and wheeze; strenthened by sesitization measurements	Fish intake decreased the risk of asthma and wheeze
Takaoka et al. 2008 (81)	n = 153; Mean = 21 yr; Japan	Cross-sectional	Age, current smoking, heredity	Self-reported doctor-diagnosed asthma, "asthma symptom score" based on 8 asthma related questions; self-reported allergy to cat, dog, or pollen	Fish and seafood intake decreased the risk of respiratory infections and pollen allergy, respectively

The studies are sorted by age of the subjects. BMI = Body mass index; FFQ = Food frequency questionnaire; n-3 = omega-3; PUFAs = Polyunsaturated fatty acids. Foods as potential covariates included in the logistic regression models are not displayed. Dietary questionnaire means no specific questionnaire, such as an FFQ, for only foods.

20

Two systematic reviews of maternal intake of long-chain n-3 PUFAs and allergies in the offspring were recently made (105, 106), one meta-analysis of observational studies and randomized controlled trials evaluating maternal intake during pregnancy (105) and one Cochrane review of randomized controlled trials evaluating maternal supplementation during pregnancy and lactation (106). As concluded from both reviews, there was not enough evidence to uniformly claim that maternal intake of long-chain n-3 PUFAs protects against allergy development. These findings are in line with the findings of a systematic review made a few years earlier on the risk of atopy in relation to early exposure of fish and long-chain n-3 PUFAs, including both maternal intakes and early child intakes (107). However, in all five studies included in which maternal intake of fish during pregnancy was investigated, an inverse association was found between maternal fish intake during pregnancy and allergy in the offspring (107). The intake of fish in childhood was less consistent, although beneficial effects were observed in nine of the 14 studies included; no associations were found in three of the studies and positive associations were found in two (107).

Taking the results of these three systematic review articles into account, the potential protective effect of fish may not only be a result of its content of n-3 PUFAs, and other factors may come into play. This notion is supported by the results of two studies showing beneficial effects of both oily and lean fish (54) or foremost of lean fish (22).

Vitamins and minerals

In addition to containing long-chain n-3 PUFAs, fish also contains substantial amounts of vitamin D and selenium (108). A diet rich in fish and foods containing vitamin D was identified as 'suggestive of benefit' in a systematic review from 2014 of food-based approaches to affect atopic diseases in childhood by maternal diet during pregnancy and lactation (109). These findings were in accordance with a systematic review and meta-analysis from 2011, including studies evaluating nutrients and foods for the primary prevention of asthma and allergy (110). Weak, and partly contradictory, but supportive evidence was found of an inverse association between high maternal intake of vitamin D during pregnancy and the development of wheeze in offspring. However, no other allergic outcomes were associated with vitamin D intake (110). Also, the association between selenium and allergy was evaluated in the diet and blood and/or urine of both pregnant mothers and in early infancy. A few weak inverse associations were observed, although they were not supportive enough to render a conclusion of a protective effect (110). In the same review, weak suggestive inverse associations were also found between asthma and zinc (110), which are found in moderate amounts in fish (108).

In addition to the above mentioned nutrients, fish contain other components, such as taurine and various peptides, and probably many yet unidentified substances that have not been evaluated in the context of immune maturation and allergy development.

MATERIALS AND METHODS

This thesis is based on results of the prospective FARMFLORA birth cohort and includes five studies that cover maternal diet during pregnancy and lactation, the fatty acid composition of breast milk during lactation as well as in in infant sera at birth and during lactation, and the children's diet up to the age of one year—in relation to farm residence and allergy development.

THE FARMFLORA BIRTH COHORT

The FARMFLORA birth cohort was initiated to investigate farm related factors associated with allergy development that may be part of the allergy protective effect of growing up on farms. The birth cohort is still being followed-up and includes 28 farm children from Skaraborg County in southwestern Sweden and 37 control children in the same rural area. Recruitment of pregnant mothers occurred at maternity clinics between September 2005 and May 2008. Recruitment of farming families was a limiting factor; all families eligible to be included in the farming group, that agreed to participate, were included. No data were collected from the farming or control families who declined to participate. Children born within the span of gestational weeks 36-42 were included in the study. To be included in the farming group, at least one parent had to work full-time or part-time on a dairy farm. Families from other types of farms or from urban areas were excluded. Written informed consent was provided by both parents, and the study was approved by the Regional Ethics Committee in Gothenburg (No. 363-05).

The FARMFLORA birth cohort was primarily designed to relate the maturation of the immune system to the microbiota, serum fatty acid composition and dietary pattern of farm and control children. A secondary aim was to relate allergy development to the same variables. The size of the study sample was based on the preceding ALLERGYFLORA birth cohort, in which significant associations between continuous bacteriological and immunological parameters were observed in 60 children (111).
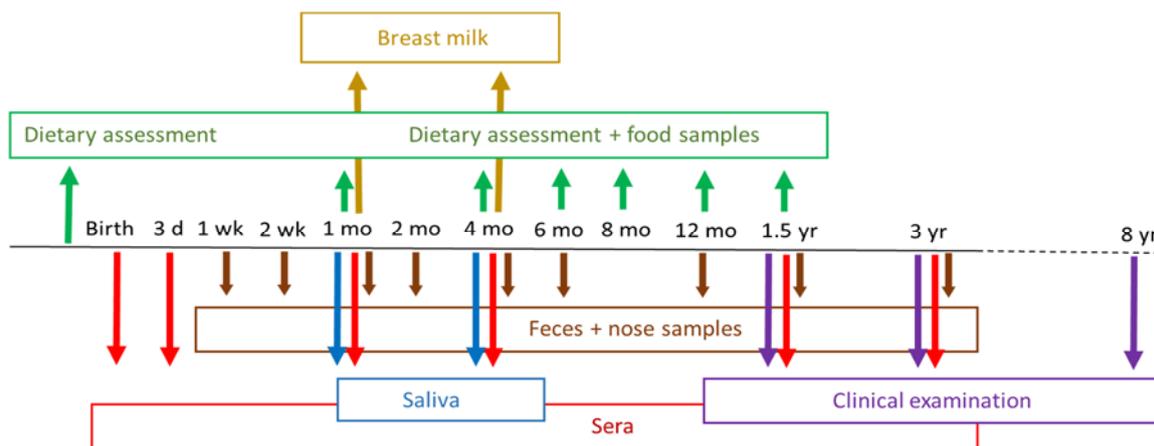

Figure 3. Sampling throughout the FARMFLORA birth cohort.

Information on dietary habits and timing of the introduction of complementary foods, as well as samples of food, breast milk, blood, saliva and microbiota from the gut and nose, have been collected throughout the study period (Figure 3). The food samples were collected for nutritional and bacteriological analyses. Samples of feces and saliva, and from the nose, were collected for

analysis of the microbiota. Blood and breast milk were sampled for analysis of for example fatty acid composition.

This thesis comprises analyses of maternal diet during pregnancy and the fourth month of lactation (Paper I), fatty acid analyses in breast milk (Paper I) and serum at birth (Paper III) and four months after birth (Paper II), and the children's diet at one year of age (Paper IV) and during weaning (Paper V; Figure 4).
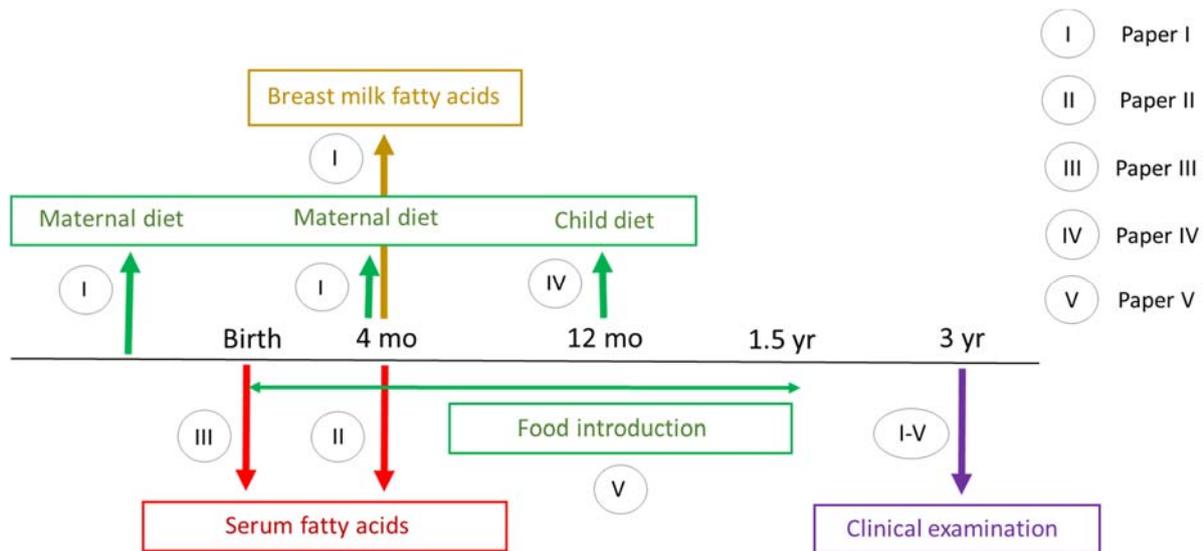

Figure 4. Sampling in the FARMFLORA birth cohort in this theses.

Subjects

One family (farmers) withdrew early from the study due to difficulties in providing study material, resulting in 28 farm and 37 control children being included in the study, of whom two farm and two control children were twins; all 4 children were included as subjects. After one and a half years, another two families withdrew, one from each group, due to a change of residence or personal issues.

At the time of recruitment, an extensive questionnaire was completed by the parents including a wide range of demographic parameters that may be related to allergy development, such as mode of delivery, gender, parental heredity, family structure and pets. Table 5 displays a selection of demographic data for farm and control children.

DIETARY ASSESSMENT

Four different dietary assessment methods were used during the different time points of assessment of the children's and their mothers' diet. During pregnancy, a semi-quantitative food frequency questionnaire (FFQ) was used to capture the mothers' diet (Paper I), while a 24-hour dietary recall, immediately followed by a 24-hour food diary, were used to assess their diet during lactation (four months postpartum, Paper I) as well as to assess the children's diet at one year of age (age range = ten to 14 months, Paper IV). To map the introduction of complementary foods, diaries were continuously kept by the families up to the children's ages of one and a half years (Paper V).

Table 5. Characteristics of farm versus control children.

	Farmers (n = 28)	Controls (n = 37)	P [e]
Antenatal characteristics			
Heredity[a]			
Mothers	7 (25%)	11 (30%)	0.68
Fathers	1 (4%)	12 (32%)	0.01
Maternal age at delivery, year	33 (21–42)	32 (22–41)	0.46[f]
Education, level[b] (1 = lowest, 5 = highest)			
Mothers	2 (1–5)	4 (1–5)	0.20[f]
Fathers	2 (1–5)	2 (1–5)	0.02[f]
Smoking during last mo of pregnancy			
Mothers	0 (0%)	1 (3%)	1.00
Fathers	1 (4%)	4 (11%)	0.38
Cats or dogs in house at recruitment	21 (75%)	19 (51%)	0.054
Siblings	18 (64%)	17 (46%)	0.15
Birth characteristics			
Gestational wk[c]	40 (37–42)	39 (36–42)	0.13[f]
Cesarean section	3 (11%)	7 (19%)	0.50
Birth weight[d], g	(2780–4740)	(2440–4830)	0.78[f]
Infant characteristics			
Male gender	10 (36%)	23 (62%)	0.04
Maternal fish oil intake, 4 mo postpartum	2 (11%)	0 (0%)	0.18
Intake of supplements of vitamin A + D at 1 yr of age	17 (61%)	22 (60%)	0.92
Allergic at 3 yr of age	1 (4%)	10 (32%)	0.02

Data are presented as *n* (%) or medians (ranges).

[a]Doctor's diagnosed asthma, rhinitis or atopic eczema.

[b]1 = Elementary school, 2 = upper secondary school 2-3 yr or equivalent, 3 = qualified graduate from upper secondary engineering course, 4 = university ≤ 1 yr, 5 = university > 1 y.

[c]*n* = 27 farmers and 36 nonfarmers, and *n* = 43 healthy and 10 allergic subjects, respctively.

[d]*n* = 36 nonfarmers, and *n* = 10 allergic subjects.

[e]χ2-Test (or Fishers exact test).

[f]Mann-Whitney *U* test.

Mothers' diet during pregnancy

Within a month after delivery, the mothers were asked to report their food intake during pregnancy in a semi-quantitative FFQ especially developed to evaluate fat and carbohydrate quality. The FFQ was based on the validated Northern Sweden 84-item FFQ (112) and extended with approximately twice as many food items to capture details about e.g. fat providing foods (113). Frequencies were reported on a nine-level scale, including never or seldom, once a month, twice a month, once a week, two to three times a week, four to six times a week, once a day, two to three times a day, and up through four times a day. Conversion to grams was based on reported estimated portion sizes. When portion sizes were not reported, standard portion sizes supplied by the Swedish National Food Agency were used (114).

An FFQ is a convenient way of measuring food patterns over a longer period of time, such as the pregnancy period. As for all announced assessment methods, there is a risk of both conscious and unconscious overestimation of healthy foods, such as vegetables and fruits, and underestimation

of unhealthy foods, such as sweets and pastries. In this thesis, we focused on fats, dairy, fish and fatty pork; in the light of current recommendations, the intake of butter, full-fat dairy and pork may be underestimated, while the intake of fish may be overestimated. As an attempt to diminish a negative self-consciousness, no questions were asked about body weight.

The specific FFQ used in our study (113) was not validated, and hence we do not know how the addition of food items may have affected the validity of the original FFQ (112). An increased number of food items and a higher level of detail may increase the respondent burden and pose a risk of over estimation of the total intake of certain food groups. However, 57 of 65 mothers completed the FFQ (88%), which is a fairly good response rate. As for the risk of over-estimation of food items, this did not appear to be the case, since the estimated intakes of the food groups we focused on were similar using both FFQs and interviews followed by diaries (Paper I (115)).

Mothers' diet during lactation and children's diet at one year of age

A combination of a retrospective 24-hour dietary recall and a prospective 24-hour food diary was used to asses both the mothers' diet during lactation and the children's diet at one year of age.

The 24-hour dietary recall was performed unannounced as a telephone interview, either by one trained nutritionist or by one of two trained nurses. A modified version of the USDA five-step multiple-pass method was used (116). The interview included questions about i) all foods and beverages consumed during the last 24 h, ii) foods that are often forgotten, iii) when and where the foods and/or beverages were consumed and iv) additional information, such as amounts and brands, for all the reported foods and beverages. To shorten the interview and reduce the burden on the respondent, the fifth step of the original protocol was omitted, i.e. the reproduction by the interviewer of all recorded food items, giving the interviewee a chance to remember any forgotten foods consumed by the mother and child. Also, household measures were used instead of food model booklets to estimate amounts.

Instructions were given to initiate the 24-hour food diary immediately after the 24-hour dietary recall. Participants were encouraged to maintain their regular dietary habits and to write down everything consumed during the 24 next-coming hours, to specify amounts and brands, and to keep an extra focus on the type of fat used. The nutrient composition of the mothers' diet during lactation was analyzed by one nutritionist, using Diet 32 (Aivo AB, Stockholm, Sweden; April 11, 2008), while the nutrient composition of the one-year-old children's diet was analyzed by one dietician, using Dietist Net Pro (Kost och Näringsdata, Stockholm, Sweden; November 2, 2015). Both softwares provided information about energy and nutrients based on the food composition database from the Swedish National Food Agency.

The dietary assessments were incomplete or missing for a number of mothers. Subjects completing at least one of the assessments were included; in these cases, the food intake reflected 24 instead of 48 hours. Twelve of the 65 mothers (18%) no longer breastfed their child at four months post-partum, hence, their diet was not assessed. Of the 53 remaining mothers who breastfed to some extent, 83% completed at least either the interview or the diary; 47% completed both, 32% only the interview, and 4% only the diary. Among the mothers to one-year-old children, 100% responded to at least one of the assessments; 74% completed both, 14% only the interview and 12% only the diary.

The interviews were unannounced in order to avoid dietary modifications caused by the dietary registration. However, this retrospective approach may cause errors in terms of lack of memory of the intake of certain foods. On the other hand, the subsequent food diary was a prospective

measure of the diet, which made it possible to capture the diet during 24 hours in detail, although at the expense of the risk of healthier choices than usual regarding diet of mother and child. To reduce self-consciousness that may influence the usual diet, no questions were asked about the body weights of either the mothers or the children. Hence, energy intake could not be related to an estimated energy requirement and, therefore, no calculations were made to detect under or over reporters. For the one-year-olds, a reference weight of Swedish one-year-old children (10.25 kg (117)) were instead used to obtain a reference value of the recommended energy intake, based on current nutrient recommendations (0.33 MJ/kg, mean for boys and girls (118)). The weight of the children in this cohort at one year of age was asked for in retrospect, approximately seven years later, and reported to be 10.29 kg in average (response rate: 58%), which coincided well with the reference value of 10.25 kg.

Introduction of formulas and complementary foods

Practices of breastfeeding, formulas and complementary foods were continuously recorded in diaries by the parents and collected at six, 12 and 18 months of age of the child. Length of exclusive and any breastfeeding was registered in months, and month of introduction of any of the following types of formulas was recorded: milk-based, gluten-based, milk-free and gluten-free formula. Month of introduction was registered for foods containing any of the following food groups: potatoes, vegetables, fruits, berries, nuts, peanuts, legumes, eggs, fish, meat, milk (dairy) and flour. A measurement of early food diversity was calculated as number of food groups introduced at the age of six months.

BREAST MILK AND SERA

Sampling of breast milk and blood

The fatty acid composition of the total lipid fraction in breast milk and cord sera was analyzed in Papers I and III, respectively, and the fatty acid composition of the phospholipid fraction in sera at four months of age was analyzed in Paper II. Dietary intakes during the past six to 48 hours may affect the fatty acid composition of breast milk (119), although the fatty acid composition in part also may be determined by a long-term intake of fatty acids that have been incorporated and stored in the fat tissue. The phospholipid fraction in sera reflects the dietary intake of fatty acids during approximately the past two to four months. The rate of samples obtained are presented in Table 6. Approximately 5 ml of breast milk was expressed manually four months postpartum, in conjunction with any of the daytime breastfeeding meals. Samples were frozen (-20°C) immediately at home in sterile plastic tubes provided by us. The samples were transferred to storage at -80°C within six months after collection. Sera was collected at birth (cord blood) and when the child was four months old; the samples were centrifuged and immediately frozen in aliquots and stored at -80 °C until analyzed.

Table 6. Sampling of breast milk and child sera in 4-month-old farm and control children.

	All subjects n = 65	Farmers n = 28	Controls n = 37
Any breastfeeding at 4 months of age	51 (78%)	22 (79%)	29 (78%)
Breast milk [a]			
Breast-milk samples, ratio of subjects breastfed to any extent	34 (67%)	15 (68%)	19 (66%)
Breast-milk samples, ratio of all subjects	34 (52%)	15 (54%)	19 (51%)
Sera			
Cord blood	54 (83%)	25 (89%)	29 (78%)
At 4 months of age[a]	50 (77%)	21 (75%)	29 (78%)

Data are presented as n (%).

[a]Due to limited amounts of breast-milk and serum samples, and the use of 2 different columns analyzing 18- and ≥20-carbon fatty acids, a number of samples were not analyzed for 18-carbon fatty acids; Breast milk: *n* = 14 Farmers and 17 for controls; Sera: *n* = 18 Farmers and 26 Controls.

Fatty acid analysis in breast milk and serum

Fatty acids in serum and breast milk samples were first extracted with chloroform and methanol according to Lee et al. (120). For serum samples collected four months post-partum, the phospholipid fraction was separated with solid phase extraction on aminopropyl solid phase extraction columns (121). After extraction (and separation), the fatty acids were methylated with direct transesterification according to Lepage and Roy (122). After methylation, the fatty acids were separated with gas chromatography and detected with flame ionization (breast milk samples and four month sera) or with mass spectrometry (cord serum samples). For the breast milk samples and four month sera, two different columns were used: a DB-WAX column for the separation of fatty acids with 16-18 carbon atoms and a HP Ultra 1 silicon column for separation of fatty acids with 20-22 carbon atoms. For analysis of fatty acids in the cord serum samples only one column, a VF-WAXms, was used. In all analyzes the fatty acids were expressed as area percentage of total fatty acids. For details on laboratory procedure or analytical setup see Papers I, II and III.

CLINICAL EXAMINATION

The children were examined at the age of one and a half and three years to diagnose food allergy, eczema, asthma and allergic rhinoconjunctivitis. Clinical diagnoses were made by trained pediatricians. The home addresses of the children were available to the pediatricians, hence they were not blinded to the children's status of being from farms or control households. However, each of the protocols was discussed with a pediatric allergologist who confirmed the diagnosis. Blood tests (Phadia, Uppsala, Sweden) were used to assess sensitization against inhalant allergens (Phadiatop) and common foods (Fx5 Food Mix).

Atopic eczema was diagnosed based on the criteria of Williams (123) or Hanifin and Rajka (124). Food allergy was defined as immediate or late-onset reactions that improved rapidly after allergen elimination, supported by open food challenge and/or Fx5 Food Mix tests (Phadia); ImmunoCAP tests (Phadia) were used to identify the allergen.

If wheezing started before two years of age, asthma was diagnosed based on ≥ three wheezing episodes combined either with eczema, allergic rhinoconjuctivitis, food allergy or wheezing between colds, or combined with a clinical response to leukotriene antagonists or inhaled glucocorticoids. At least one wheezing episode had to have occurred after the age of two years. The same criteria were used if wheezing started after two years of age, except that one wheezing episode (instead of three) was required.

Mandatory symptoms for a diagnosis of allergic rhinoconjunctivitis were symptoms from eyes or nose after exposure to pollen or animal dander, combined with a demonstration of allergen-specific IgE test to the corresponding inhalant allergen (ImmunoCAP, Phadia).

Children that were not allergic did not have any other chronic disease and were all classified as healthy.

Table 7. Matenal dietary data during pregnancy and lactation and sampling of breast milk and child sera in 4-month-old healthy and subsequently allergic children.

	All subjects n = 55	Healthy n = 44	Allergic n = 11
Any breastfeeding at 4 months	44 (80%)	36 (82%)	8 (73%)
Maternal diet			
Pregnancy[a]	50 (92%)	39 (88%)	11 (100%)
Lactation[b], ratio of subjects breastfed to any extent	38 (86%)	31 (86%)	7 (88%)
Lactation[b], ratio of all subjects	38 (69%)	31 (70%)	7 (63%)
Breast milk[c]			
Breast-milk samples, ratio of subjects breastfed to any extent	30 (68%)	25 (69%)	5 (63%)
Breast-milk samples, ratio of all subjects	30 (55%)	25 (57%)	5 (45%)
Sera			
Cord blood	47 (85%)	37 (84%)	10 (91%)
At 4 months of age[c]	41 (75%)	32 (73%)	9 (82%)

Data are presented as n (%).

[a] Food frequency questionnaire.

[b] 24-h recall combined with 24-h diary. Numbers represents completion of either the recall or the diary or both.

[c] Due to limited amounts of breast-milk and serum samples, and the use of 2 different columns analyzing 18- and ≥20-carbon fatty acids, a number of samples were not analyzed for 18-carbon fatty acids; Breast milk: *n* = 23 Healthy; Sera: *n* = 27 Healthy and 8 Allergic.

In this thesis, any allergic manifestation (atopic eczema, asthma, food allergy or allergic rhinoconjunctivitis) at three years of age was used as the endpoint (Paper I-V). Children diagnosed with an allergy at one and a half but not three years of age were excluded: seven children (two farm and five control children). In addition, one child from the farming group did not undergo clinical examination at one and a half years. These children were included neither in the healthy group nor the group with allergy diagnosed at three years of age. Thus, considering the two families that withdrew from the study owing to a change of residence or personal reasons, 55 children were eligible for analyses of differences between healthy (*n* = 44) and allergic (*n* = 11) individuals at three years of age. As previously reported, one of 28 farm children (4%) and ten of 37 control children

(32%) developed allergy at three years of age (125). Any allergy was used as outcome, due to the limited number of allergic children. The response rate regarding the mothers' diet during pregnancy and lactation as well as the rate of samples of breast milk and sera obtained for healthy and allergic children are presented in Table 7.

STATISTICAL ANALYSIS

Multivariate analysis

Principal Component Analysis (PCA) is an unsupervised multivariate method used to display the distribution of subjects in the multivariate space, based on differences in several, usually a large set, of variables (Paper II). Orthogonal Projections to Latent Structures with Discriminant Analysis (OPLS-DA), which was used in Papers I, II, IV and V is a regression variety of PCA and is a supervised method that maximizes the separation between subjects according to pre-determined groups (here, Farmers vs. Controls and Healthy vs. Allergic), based on the variables entered into the model (126, 127). An OPLS-DA scores plot visualizes the distribution of subjects, i.e. the separation of the different groups. An OPLS-DA *loadings* plot displays the contribution of each variable to the separation of the groups. A large number of variables in a moderate number of observations can be analyzed using OPLS and PCA; the variables need neither to be normally distributed nor independent of one another.

Univariable analysis

Due to small sample sizes and non-normal, often skewed distributions of the variables, nonparametric tests were used and data were presented as medians and interquartile ranges, except for demographic data that were presented as medians and ranges for continuous data and number and as percentages for categorical data. Categorical variables were analyzed using the χ^2 test or Fisher's exact test and continuous variables using the Mann-Whitney U test. Spearman's *rho* was used for calculation of correlations (Papers I, II, II and IV), while trends were analyzed by the χ^2-test or Fisher's exact test (Paper V). Two-tailed P-values ≤0.05 were used as the limit of significance in all papers; exceptions were for correlations between long-chain n-3 PUFAs in serum and breast-milk and maternal intake of oily fish; then one-tailed P-values were considered due to well established evidence for positive correlations (Paper II).

Multivariable analysis

Logistic regression was used for multivariable analysis (Papers I-V). Due to the limited size of the allergic group, potential covariates were entered independently in separate models. In the adjusted models, variables were included as covariates if $P \leq 0.2$ in univariable analysis of healthy versus subsequent allergic children.

Stratification

As allergic children were almost exclusively found in the non-farming control group, differences between allergic and healthy infants could merely be a reflection of the differences between the farming and control families' diets. To adjust for the 'farm effect', analyses were made in the control group, in addition to analyses in the entire cohort (Papers I, II, IV and V).

Reverse causation

To adjust for reverse causation in Paper V, a question was sent in retrospect by mail to all families: 'Did you wait deliberately to introduce certain foods to the child (e.g. fish, eggs, flour/gluten) due to allergy in the family or early signs of allergy in the child?' Fifty-five children were eligible for analyses between healthy children and children allergic at three years of age. Seventy-one percent of those families responded to the question regarding reverse causation including 8 of the 11 allergic children, and all responded negatively despite a family history of allergy in almost half of the responding families. Hence, we did not adjust for reverse causation.

RESULTS

DIFFERENCES BETWEEN THE FARM AND CONTROL GROUPS

Differences in diet between farm and control mothers during pregnancy and lactation, focusing on fatty foods and fatty acids

The diet of farm and control mothers was assessed during pregnancy and four months post-partum using an FFQ and a 24-hour dietary recall followed by a 24-hour food diary, respectively (Paper I). Orthogonal projections to latent structures with discriminant analysis (OPLS-DA) was used to examine dietary differences in food items and food groups (pregnancy and lactation) and energy and macronutrients, including fatty acids, (lactation) between farming and control mothers. Univariable significances were analyzed using the Mann-Whitney U test.
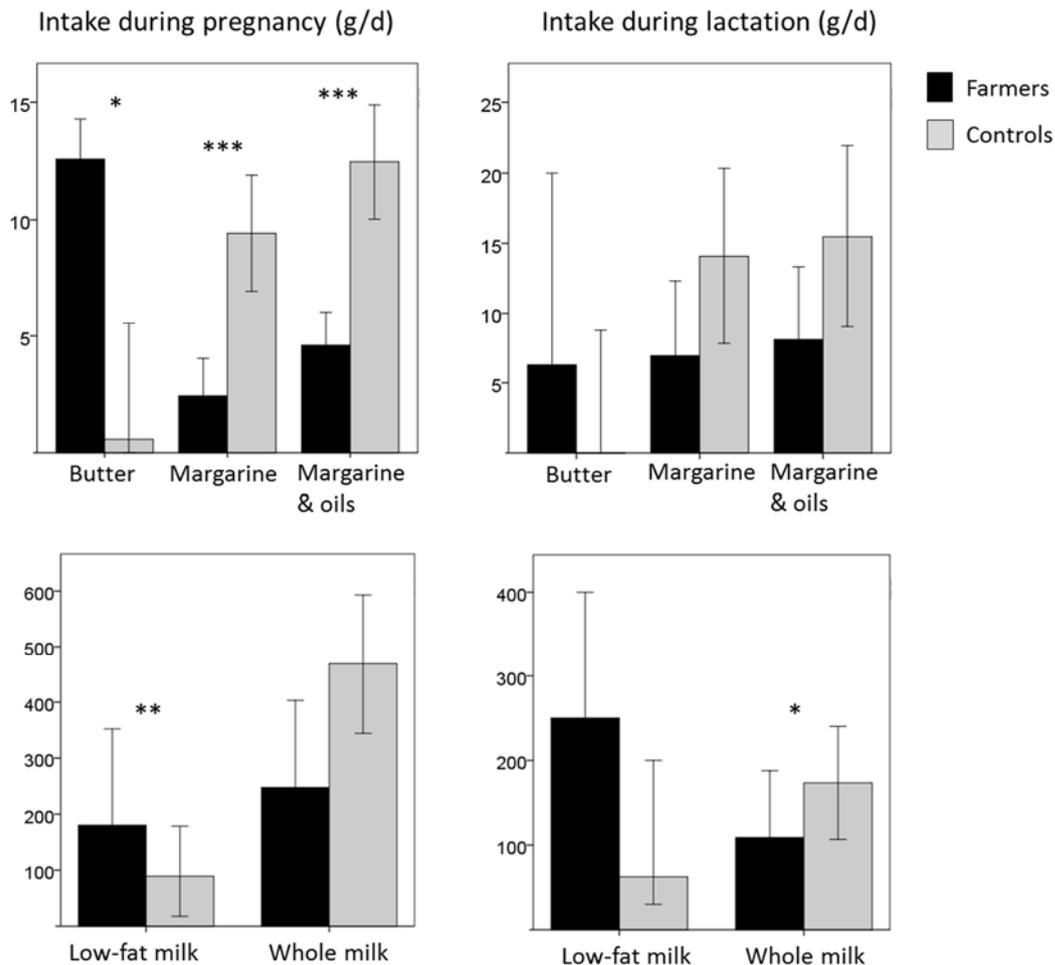
Figure 5. Intake of fats and milk of different fat quality in farm and control mothers.
Differences were analyzed using Mann-Whitney U test. Data are presented as medians and error bars denote the 95% confidence interval. The stars denote the significance level of: *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$.

A pattern of higher intakes of full-fat dairy and saturated fatty acids, and lower intakes of margarines and oils, was observed in the farming group, during both pregnancy and lactation (Paper I). The percent of energy derived from saturated fat (18 vs. 15%, $P = 0.01$) and total fat (37 vs. 33%, $P = 0.03$) was higher in farmers, as was the absolute intake of saturated fatty acids (10 vs. 8 g/d, $P = 0.03$). Medians of the intake of margarine, margarine and oils, butter, low-fat milk and whole milk in farming and control mothers, and P-values of the differences between the groups, are displayed in Figure 5.

Fatty acid composition of breastmilk and infant sera in farm and control children

Fatty acids in breast milk of farming and control mothers

Breast milk was collected four months post-partum, and a range of foremost saturated and polyunsaturated fatty acids was analyzed in farming and control mothers (Paper I). Farming mothers had significantly higher proportions of saturated fatty acids in their breast milk, compared to control mothers, who instead had significantly higher proportions of the n-6 PUFA linoleic acid and the n-3 PUFA alpha-linolenic acid in their breast milk (Fig. 6).
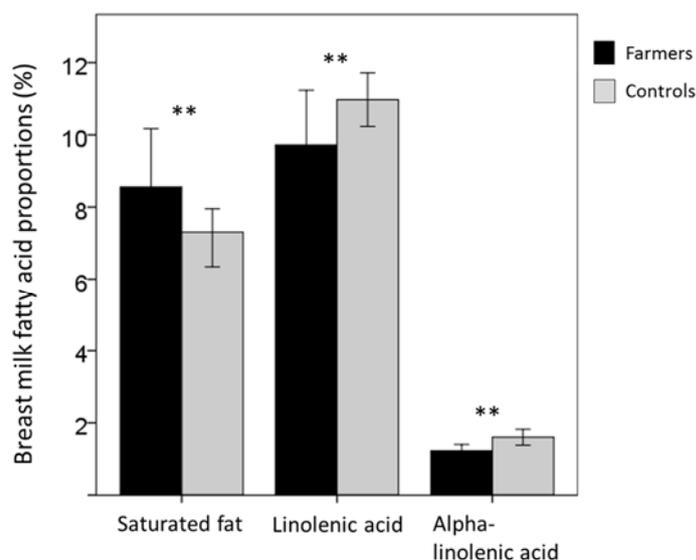
Figure 6. Differences in fatty acid composition of breast milk among farm and control mothers. Differences were analyzed using Mann-Whitney *U* test. Data are presented as medians and error bars denote the 95% confidence interval. The stars denote the significance level of: **$P \leq 0.01$.

Fatty acid composition of serum in farm and control children

Serum samples of the infants were obtained at birth (Paper III) and at four months of age, (Paper II) and fatty acid composition in the total lipid fraction and the phospholipid fraction was analyzed, respectively, in farm and control children. The serum composition at birth did not differ notably between farm and control children, except for slightly higher proportions of n-6 PUFAs, foremost arachidonic acid, in farm children. For sera at four months of age, principal component analysis was used to visualize differences in the fatty acid composition of sera between farm and control infants; also here, no substantial differences were observed. In univariable analysis, the only differences found between the children from farm and control families were higher proportions of

the monounsaturated n-7 fatty acid 18:1 in control infants (2.0% vs 1.8%, $P = 0.02$) and insignificantly higher proportions of the saturated fatty acid 18:0 in farm infants (18% vs. 16%, $P = 0.22$).

Table 8. Intakes of foods and nutrients that differed between farm and control children at 1 year of age.

	Farmers (n = 28)	Controls (n = 37)	P[d]
Food items/groups, g/d			
Butter[a]	3 (0-10)	0 (0-5)	0.08
Margarine	0 (0-3)	2 (0-6)	0.06
Farm milk[b]	0 (0-75)	0 (0-0)	0.00[e]
Cream (27-41 % fat)	1 (0-6)	0 (0-1)	0.02[e]
Oily fish	0 (0-12)	0 (0-0)	0.02[e]
Poultry	0 (0-0)	0 (0-5)	0.03[e]
Porridge/gruel, home made[c]	0 (0-43)	0 (0-0)	0.05[e]
Macronutrients, g/d			
Carbohydrates	130 (110-140)	135 (124-151)	0.02
Fat	33 (26-49)	32 (27-41)	0.01
Cholesterol, mg	85 (57-118)	60 (35-96)	0.02
Fatty acids, g/d			
4:0–10:0	1.0 (0.7-1.8)	0.6 (0.4-1.3)	0.01
14:0	1.4 (1.0-2.0)	1.0 (0.7-1.6)	0.01
18:0	2.3 (1.6-3.4)	2.0 (1.4-2.6)	0.02
Saturated fatty acids, sum	14 (9-21)	13 (10-17)	0.02
16:1	0.5 (0.3-0.6)	0.3 (0.2-0.4)	0.01
20:4, n-6	0.05 (0.02-0.07)	0.03 (0.02-0.05)	0.04
Micronutrient, µg /d			
Iodine	38 (25-66)	33 (18-50)	0.03

Data are presented as medians (interquartile ranges).

[a]Includes butter based margarines.

[b]Incudes both pasteurized and unpateurized farm milk.

[c]Wet weight.

[d]Mann-Whitney U test; P-values are shown for energy adjusted intakes.

[e]Differences are significant as both absolute and energy adjusted intakes.

Differences in the diet of farm and control children at one year of age and resemblances to their mothers' diet

Diet in farm and control children

Fourteen percent of the children were still breastfed to some extent at one year of age, five farm children and four control children; hence, both absolute and energy adjusted values were calculated of their food and nutrient intake. Children from farming families had a higher intake of farm milk, whole cream, oily fish and home-made porridge/gruel compared to control children, who instead ate more poultry. The farm children tended to consume more butter, while the margarine intake tended to be higher in control children.

The differences in nutrient intake between farm and control children were more pronounced when the diet was calculated as energy-adjusted values than absolute values. Children from farming families had higher intakes of cholesterol and the fatty acids 4:0-10:0, 14:0, 18:0 as well as saturated fat and fat in total, compared to children from control families. The intake of the fatty acids 16:1

and 20:4 (arachidonic acid) and iodine was also higher in farm children, while the control children had a higher intake of carbohydrates. Dietary differences between farm and control children are displayed in Table 8; significances are shown for energy adjusted intakes, while intakes are shown in absolute values for a better picture of the actual diet. Tables with a complete set of both absolute and energy adjusted intakes are found in Paper IV.

Resemblances in the dietary patterns of the farm children and their mothers
A question we wanted to address in this thesis was whether the dietary pattern of the one-year-old farm children resembled that of their mothers, as assessed during pregnancy and lactation four months post-partum (Paper IV). Figure 7 displays OPLS-DA loadings plots of the dietary pattern of the farm children and their mothers in relation to the dietary patterns of the respective control group; variables that characterized the diet of either the children or the mothers are shown. A pattern of more butter, full-fat dairy, saturated fat and fat in total was observed in the diet of both the farm children and their mothers, compared to children and mothers in the control group, whose diets instead were characterized by a higher intake of margarines and oils, low-fat dairy and carbohydrates (Figure 7). In addition, we assessed whether the children's intake of foods that were typical of the farming diet correlated with their mothers' intake; farm and control families were analyzed separately. In the farming group, significant correlations were found between the diet of mother and child, regarding intake of whole milk (*rho* = 0.51, P = 0.03 [pregnancy]; *rho* = 0.38, P = 0.09 [lactation]) and butter (*rho* = 0.57, P = 0.012 [pregnancy]; *rho* = 0.59, P = 0.01 [lactation]); no significant correlations were found in the diet of control children and their mothers (data not shown).

Introduction of complementary foods in farm and control children

Orthogonal projections to latent structures with discriminant analysis was used to visualize patterns in the introduction of complementary foods between farm and control children (Paper V). Both nuts and peanuts were observed to be introduced earlier in the farming families than in the control families, while the first introduction of formula or any foods occurred later, i.e. exclusive breastfeeding was of a longer duration in the farming group. In univariable analysis, only the earlier introduction of nuts in farm children was significant (11 vs. 15 months, P = 0.02), although the introduction of peanuts tended to occur earlier (19 [IQR: 13-19] vs. 19 [IQR: 19-19] months, P = 0.07) and the duration of exclusive breastfeeding tended to be longer (4.0 vs. 3.5 months, P = 0.11).
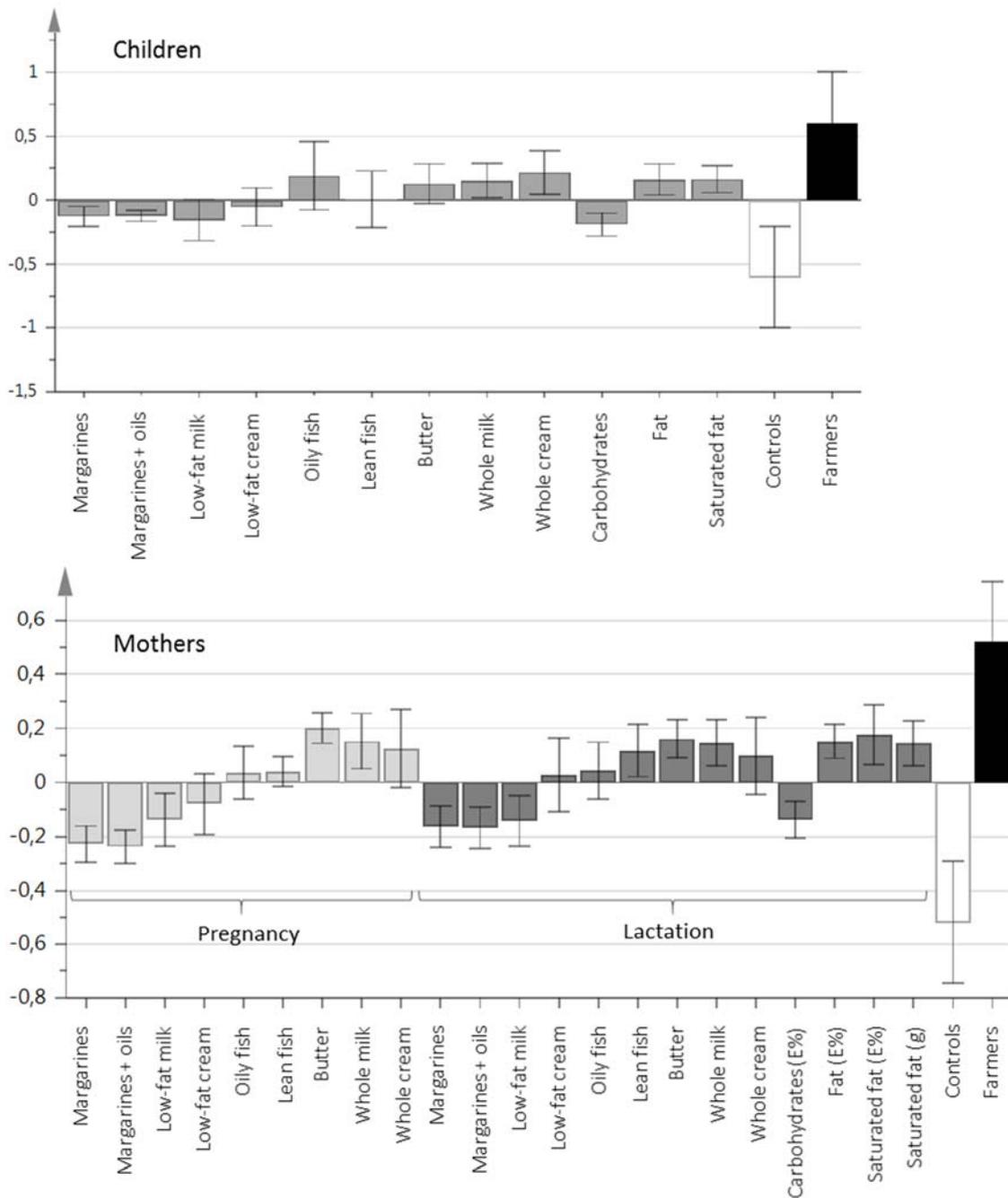
Figure 7. Dietary patterns in farm children and their mothers in relation to controls.
Differences between farmers and controls are displayed in an orthogonal projections to latent structures with discriminant analysis (OPLS-DA) loadings plot. Variables pointing in the same direction as farmers are higher in farmers and vice versa; the higher the bar the larger the difference between the farmers and the controls. Error bars denote the 95% confidence level. Two components were used for the model for the children; R^2X(cum) = 0.43 and Q^2(cum) = 0.20. One component was used for the model for the mothers; R^2X(cum) = 0.24 and Q^2(cum) = 0.28.

DIFFERENCES BETWEEN HEALTHY AND SUBSEQUENTLY ALLERGIC CHILDREN

Allergy diagnoses

Of the 65 children included at baseline, 11 were diagnosed as allergic at the age of three years, ten in the control group of 37 children (32% allergy prevalence) and only one in the farming group of 28 children (4% allergy prevalence). This marked difference in allergy prevalence between farming and control children was previously reported by Strömbeck *et al.* (125). Considering only those 55 children that were eligible for analyses between healthy and allergic children (due to e.g. dropping out or unclear allergy diagnoses), still 4% of the farm children (one of 24) and 32% of the controls (ten of 31) were allergic. The distribution of allergy diagnoses among the allergic children is shown in Table 9. We aimed to relate the pregnant and lactating mothers' diet (Paper I), the fatty acid composition of breast milk (Paper I) and children's sera at birth (Paper III) and at four months of age (Paper II), the introduction of complementary foods (Paper V) and the one-year-old children's diet (Paper IV) to allergy at the age of three years.

Table 9. Allergy diagnoses[a] at 3 years of age.

	Eczema	Asthma	Food allergy	ARC[f]
Farm children				
1	x			
Control children				
1	x			
2[b]	x			
3[b]	x			
4[c]	x			
5[cd]	x		x	
6[c]	x	x		
7[cd]		x		
8[cd]		x		
9[cd]		x		x
10[e]			x	

[a]Diagnosed by trained pediatricians, using strict predefined protocols.
[b]No serum samples provided at 4 months.
[c]No breast-milk samples provided.
[d]No assessment of maternal diet during lactation provided.
[e]No cord serum samples provided.
[f]Allergic rhinoconjunctivitis.

Differences in diet between mothers of healthy and subsequently allergic children during pregnancy and lactation, focusing on fatty foods and fatty acids

The dietary pattern of the mothers was examined by OPLS-DA, which revealed higher intakes of margarines, oils and low-fat dairy both during pregnancy and lactation in mothers of allergic children, compared to mothers of healthy children. Since only one farm child was included in the allergic group, differences between healthy and allergic children could simply be a reflection of differences between farmers and controls. To address this problem, dietary differences between mothers of healthy and allergic children were examined in the control group separately. The

distinctly higher intake of margarines and margarines plus oils in the mothers of allergic children remained in the OPLS-DA model of controls. Median intakes of margarines and margarines and oils in mothers of healthy and subsequent allergic children, and *P*-values of the differences between the groups, are presented in Figure 8.
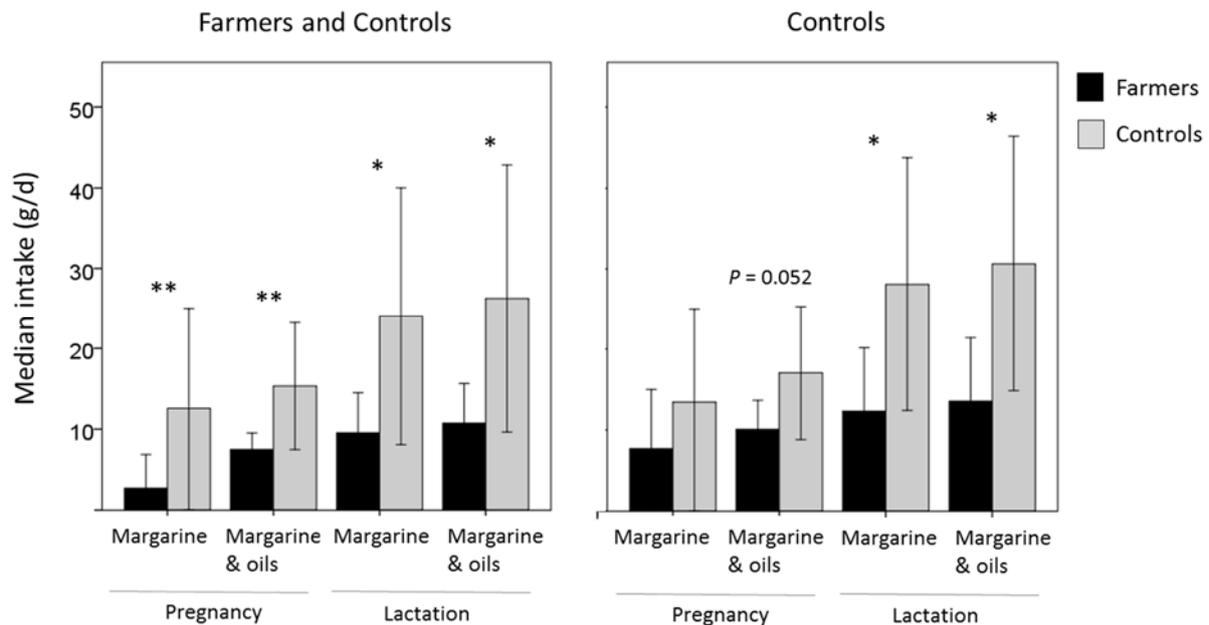

Figure 8. Intake of margarines and oils during pregnancy and lactation in mothers of healthy and subsequently allergic children.
Differences were analyzed using Mann-Whitney *U* test. Data are presented as medians and error bars denote the 95% confidence interval. The stars denote the significance level of: *$P \leq 0.05$ and **$P \leq 0.01$.

Logistic regression with margarine and oil intake during pregnancy or lactation as explanatory variables and allergy as outcome was performed in controls. Potential covariates from Table 5 (characteristics) were included in separate models if the *P*-value for differences between mothers of healthy and subsequent allergic control children was below or equal to 0.2 in univariable analysis. The crude odds ratio (OR) for every portion of margarines and oils consumed per day (one portion = 5 g, Swedish National Food Agency, 2001) was 1.91 (95% CI: 1.02-3.56, *P* = .04) during pregnancy and 1.50 (95% CI: 1.02-2.21, *P* = .04) during lactation. The ORs did not decrease substantially in any of the adjusted models, suggesting little influence of covariates.

Fatty acid composition of breast milk and infant sera in healthy and subsequently allergic children

Breast milk composition in mothers of healthy and subsequently allergic children
The breast milk of mothers to healthy children contained higher proportions of the long-chain n-3 PUFA docosahexaenoic acid (DHA; 0.2% vs. 0.1%, *P* = .03) and the saturated fatty acid 18:0 (7.8% vs. 6.8%, *P* = .04), compared to the breast milk of mothers of allergic children, which contained higher proportions of the monounsaturated fatty acid 18:1 n-9 (39% vs. 36%, *P* = .04). After exclusion of farmers, the higher breast milk proportions of DHA in mothers of healthy children tended to remain but was no longer significant (0.3% vs. 0.2%, *P* = .09).

Fatty acid composition of serum in healthy and subsequently allergic children
Serum proportions of EPA differed markedly between healthy and subsequently allergic children, both at birth and at four months of age, although most markedly at four months of age. The EPA proportions were unrelated to farming status. The sum of long-chain n-3 PUFAs also differed significantly at four months of age, and the proportions of DHA tended to be higher in healthy children. Figure 9 displays differences in serum fatty acid proportions of EPA, long-chain n-3 PUFA and DHA at four months of age between healthy and subsequently allergic children, both in all subjects and controls only.

Healthy children also had higher serum proportions of the saturated fatty acid 18:0 at four months of age (17% vs. 16%, $P = 0.03$) and tended to have lower proportions of the n-6 PUFA linoleic acid compared to subsequently allergic children (20% vs. 21%, $P = 0.06$). However, the higher proportions of 18:0 in the healthy children's sera did not remain significant in controls only (16% vs. 16%, $P = 0.11$); while instead the tendency toward lower proportions of linoleic acid became significant (20% vs. 21%, $P = 0.02$).
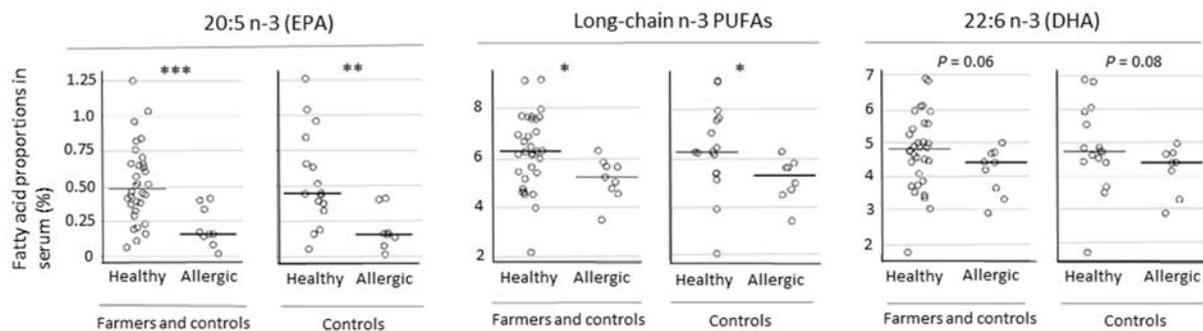
Figure 9. Proportions of long-chain n-3 polyunsaturated fatty acids in sera of healthy and subsequently allergic children.
Differences were analyzed using Mann-Whitney *U* test. The horizontal bar denote the median value of the group. The stars denote the significance level of: *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$.
Abbreviations: DHA = docosahexaeonic acid; EPA = eicosapentaenoic acid; PUFAs = polyunsaturated fatty acids; n = omega.

Logistic regression was performed to relate the child's serum proportions of EPA to a diagnosis of allergy at three years of age. The crude OR was 0.43 (95% CI: 0.19-0.98, $P = 0.05$) for cord sera and 0.47 (95% CI: 0.27-0.83, $P = 0.01$) for sera at four months of age, for every 0.1% increase in serum EPA. Potential covariates were entered independently in separate models; the analyses included both farm and control children, since EPA in sera was unrelated to farming status. The OR for EPA changed more than 10% only in the models including exclusive or any breastfeeding; however, the ORs for EPA were still low: $OR_{birth} = 0.62$, 95% CI: 0.30-1.30 and $OR_{4mo} = 0.57$, 95% CI: 0.33-0.97, and $OR_{birth} = 0.53$, 95% CI: 0.24-1.10 and $OR_{4mo} = 0.52$, 95% CI: 0.29-0.93, respectively.

Correlations between allergy, fatty acids in serum, maternal diet and breast milk

The proportions of EPA in serum both at birth and at four months of age correlated with the mothers' intake of oily fish during pregnancy (*rho* = 0.33 and 0.42, $P = 0.02$ and 0.01, respectively). An overview of correlations between allergy and proportions of long-chain n-3 PUFAs in the sera

at four months of age, proportions of the corresponding fatty acids in breast milk and maternal intake of oily fish during pregnancy and lactation are shown in Figure 10. High serum proportions of EPA were associated with lower odds of developing allergy at three years of age; proportions of EPA in sera correlated with proportions of EPA in breast milk, which in turn correlated with maternal intake of oily fish during lactation and tended to correlate with oily fish intake during pregnancy as well. The same pattern was observed for serum proportions of both DHA and long-chain n-3 PUFAs in total, although they were more weakly associated with allergy but correlated more strongly with the proportions of the corresponding fatty acids in breast milk. The proportion of DHA and long-chain n-3 PUFAs in breast milk correlated to the mother's intake of oily fish during both pregnancy and lactation (Fig. 10).
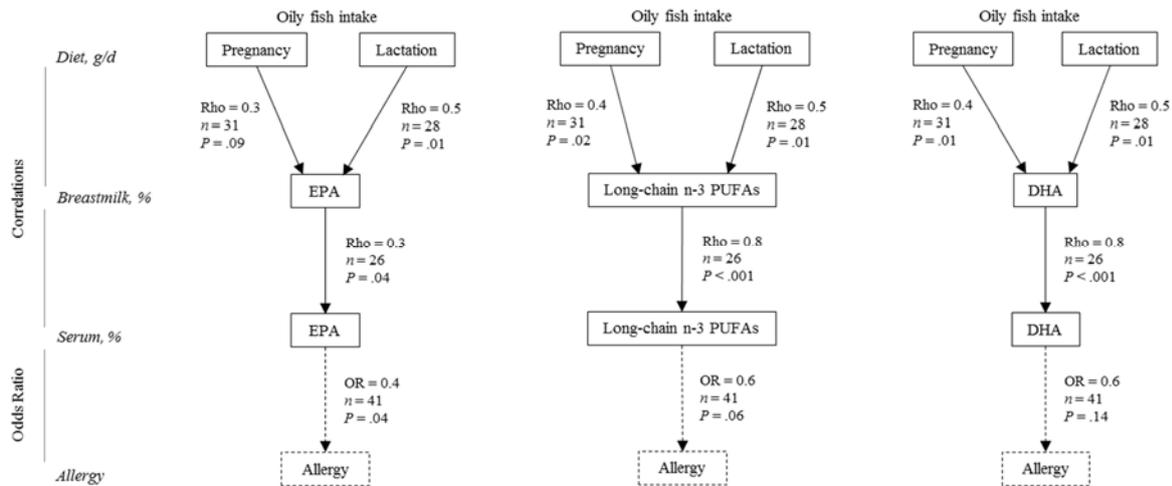
Figure 10. Overview of the relation between allergy, long-chain n-3 PUFAs in child sera and breast milk, and maternal oily fish intake during pregnancy and lactation.
The sera and breast milk was obtained four months postpartum. The OR for developing allergy was analyzed for every 0.1% increase in serum proportions of EPA, and every 1% increase for the other fatty acids. One-tailed Spearman's *rho* was used for correlations. *Abbreviations:* DHA = docosahexaeonic acid; EPA = eicosapentaenoic acid; n = omega; OR = odds ratio; PUFAs = polyunsaturated fatty acids.

Since the infants' serum proportions of EPA were strongly associated with a decreased risk of allergy in the children, we wanted to see if the serum EPA correlated to the mothers' intake of margarines and oils during pregnancy and lactation, which was associated with a weak increased risk of allergy in the children. The maternal intake of margarines and oils during pregnancy correlated negatively with serum EPA (*rho* = -0.48, P = 0.001), while the correlation between the intake during lactation and serum EPA was weak and insignificant (*rho* = -0.23, P = 0.20; unpublished data).

Introduction of complementary foods in healthy and subsequently allergic children

Differences in practices of introducing complementary foods were evaluated in paper V. Data on the month of introduction of a range of different food groups were obtained for all 65 children. In OPLS-DA, a pattern of later introduction of foremost nuts, fish, milk, peanuts, flour and potatoes was observed in subsequently allergic children, as was a lower food diversity at six months,

compared to healthy children, for whom the first introduction of formula or any food occurred later (longer duration of exclusive breastfeeding; Fig. 11).
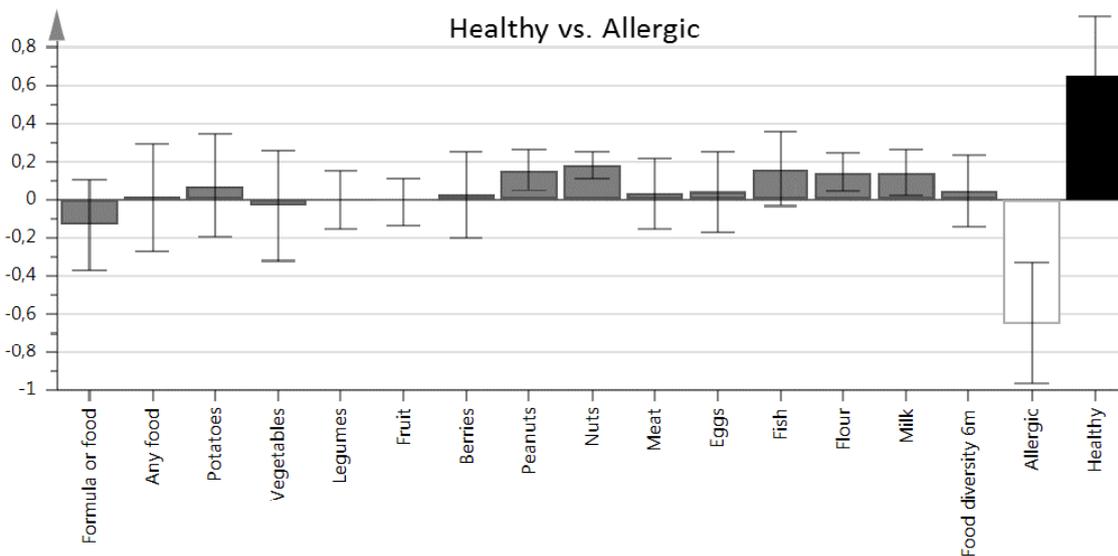

Figure 11. Food introduction practices in families of healthy and subsequently allergic children, as displayed by orthogonal projections to latent structures with discriminant analysis.
Differences between healthy and subsequently allergic children are displayed in an orthogonal projections to latent structures with discriminant analysis (OPLS-DA) loadings plot. A bar pointing in the same direction as Healthy demonstrates earlier introduction or higher food diversity in Healthy and vice versa; the higher the bar the larger the difference between the Healthy and the Allergic. Error bars denote the 95% confidence level. One component was used for the model; $R^2X = 0.13$, $R^2 = 0.21$ and $Q^2 = -0.07$.

In univariable analysis, the duration of any and exclusive breastfeeding was significantly shorter in the allergic group compared to the healthy group. When divided into months up to which breastfeeding had occurred, a lower number of allergic subjects were found only among children that were exclusively breastfed up to three months of age (Table 10). When the analysis was confined to controls, a significantly lower number of allergic children was found only up to one month of age (Table 10). Fish had been introduced at ten months of age in a significantly lower number of allergic children, although the significance was lost in controls only (Table 10). In both the entire cohort and just controls, a lower number of allergic children had received eggs at ten months of age compared to subsequently allergic children (Table 10). Moreover, the introduction of flour tended to occur earlier in healthy than subsequently allergic children. In multivariable analysis, late introduction of flour was significantly associated with allergy when adjusted for exclusive breastfeeding, although it was insignificantly associated in controls only (Table 10). The families were asked by mail in retrospect whether the introduction of any foods had deliberately been postponed. Sixty-two families returned a response and all responded negatively, despite a family history of allergy among approximately half of the families and early signs of allergy in two children among the responding families. Hence, reverse causation was not accounted for.

Table 10. Differences in introduction practices of complementary foods in healthy children and children allergic at 3 years of age.

	All subjects			Controls		
	Healthy	Allergic		Healthy	Allergic	
	(*n* = 44)	(*n* = 11)	*P*	(*n* = 44)	(*n* = 11)	*P*
Univariable analysis						
Any breastfeeding, months[a]	8 (5-12)	6 (2-7)	0.02	8 (4-12)	5 (2-7)	0.04
Exclusive breastfeeding, months[a]	4 (2-4)	1 (0-3)	0.01	4 (2-4)	0.5 (0-3)	0.02
Exclusively breastfed up to[b]:						
1 month	47%	10%	0.01	70%	14%	0.01
2 months	37%	11%	0.04	50%	18%	0.12
3 months	36%	7%	0.01	55%	45%	0.07
Introduction ≤10 months of:						
Eggs	100%	17%	0.04	100%	30%	0.32
Fish	67%	14%	0.011	80%	23%	0.03
Introduction of flour, months[a]	5 (5-6)	6 (5-6)	0.08	5 (5-5)	6 (5-6)	0.16
Multivariable analysis [c]						
Exclusive breastfeeding[d]	OR: 0.57		0.01	OR: 0.52		0.02
	(95% CI: 0.38-0.86)			(95% CI: 0.31-0.89)		
Introduction of flour[d]	OR: 1.57		0.19	OR: 2.72		0.13
	(95% CI: 0.80-3.08)			(95% CI: 0.75-9.84)		
Introduction of flour, adjusted for exclusive breasfeeding[d]	OR$_a$: 2.39		0.04	OR$_a$: 2.18		0.27
	(95% CI: 1.05-5.43)			(95% CI: 0.54-8.73)		

Data are presented as medians (interquartile ranges).

OR = odds ratio; CI = confidence interval.

[a]Mann-Whitney *U* test.

[b]χ^2-test or Fisher's exact test.

[c]Logistic regression.

[d]OR for every 1 month increase.

Differences in the diet at one year of age in healthy and subsequently allergic children

Dietary data from either the 24-hour dietary recall and/or the 24 dietary interview were collected from all of the 65 one-year-old children, and differences in the diet between healthy and subsequently allergic children were analyzed in paper IV. Although the duration of breastfeeding was longer in the group of healthy children, as compared to the group of subsequently allergic children, the difference in the rate of being breastfed at one year of age was not significant (18% vs. 9%, respectively, *P* = 0.13). Children in the healthy group consumed more seafood (75th percentiles: 10 vs. 0 g/d, *P* = 0.02) and tended to have a lower intake of pork (medians: 7 vs. 10 g/d, *P* = 0.052) than subsequently allergic children. When these analyses were confined to control children, the significance was lost regarding the healthy children's higher intake of seafood. In contrast, the lower intake of pork did, alongside with a lower intake of zinc, become significant for energy adjusted intakes. The crude OR, in control children, for every gram of seafood was 0.45 (95% CI: 0.14-1.43) and for every gram of pork 1.37 (95% CI: 1.01-1.84). The OR for seafood changed >10% when adjusted for exclusive breastfeeding, any breastfeeding (OR$_a$: 0.39, 95% CI: 0.11-1.44) and cesarean section (OR$_a$: 0.31, 95% CI: 0.08-1.30). The OR for pork, in turn, only increased when adjusted for covariates; increases of >10% were found when exclusive breastfeeding, any breastfeeding and female gender were included in the model (Paper III). Insignificant differences were found in the intake of margarines between healthy and subsequently allergic children (0.1 vs. 6.3 g/d; Paper III, data not shown). Using logistic regression in retrospect,

the OR for developing allergy at three years of age was found to be 1.25 with a 95% CI of 1.06-1.49 ($P = 0.01$) for every gram of margarine consumed at one year of age (unpublished data). The OR did not change after adjustment for potential covariates, except for a slight increase when adjusted for partial breastfeeding (OR: 1.38, 95% CI: 1.09-1.75, $P = 0.01$).

Since the higher maternal intake of margarines and oils and the lower serum proportions of EPA four months post-partum were associated with allergy, correlations were made between those variables and the intake of seafood, pork and zinc in the diet of the one-year-olds. The children's intake of seafood at one year of age was found to correlate with their serum proportions of EPA at four months of age (*rho* = 0.35, $P = 0.01$). Moreover, the children's margarine intake correlated with their mothers' intake of margarine and oil both during pregnancy (*rho* = 0.35, $P = 0.01$) and lactation (*rho* = 0.32, $P = 0.04$). No other correlations were present, except for tendencies toward negative correlations between the serum EPA and the children's intake of margarine and zinc (*rho* = -0.28 and 0.25, $P = 0.053$ and 0.08, respectively).

To give an overview of differences in the diet and fatty acid composition of breast milk and sera between the families of healthy and subsequently allergic children, an OPLS-DA loadings plot was made, based on variables that characterized the groups (Fig. 12). A consistent pattern of more oily fish in the diet of families with healthy children was observed throughout all time points of assessments, which was reflected in both breast-milk and infant sera as higher proportions of long-chain n-3 PUFAs, as compared to the families with subsequently allergic children. Also, a lower intake of margarines was observed during pregnancy and lactation in mothers of healthy children as well as in the healthy children's diet at one year of age. Higher proportions of linoleic acid were found in the healthy control children's sera at four months of age. Moreover, patterns of earlier introduction of complementary foods were found for the healthy children.
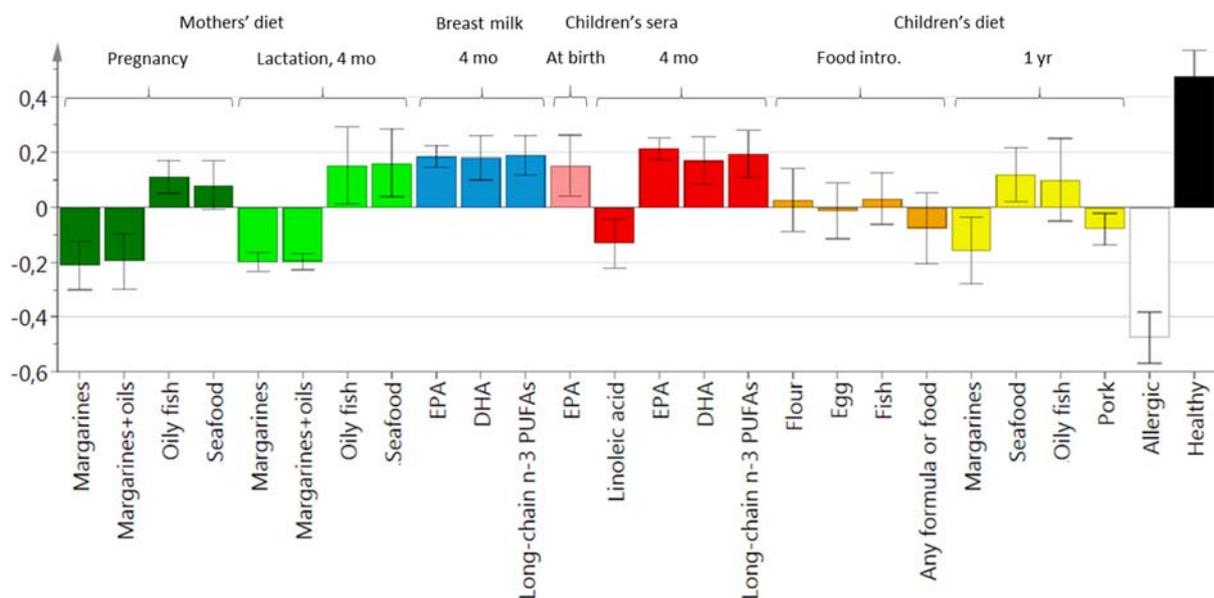
Figure 12. Overview of differences in maternal diet, children's diet and fatty acid proportions of breast-milk and infant sera between healthy and subsequently allergic children.
Differences between healthy and subsequently allergic children are displayed in an orthogonal projections to latent structures with discriminant analysis (OPLS-DA) loadings plot. A bar pointing in the same direction as Healthy demonstrates higher values of the variable, or earlier introduction of the food, in Healthy and vice versa; the higher the bar the larger the difference between the Healthy and the Allergic. Error bars denote the 95% confidence level.

DISCUSSION

A markedly low incidence of allergy, one of 28 (4%), has previously been reported among farmers' children in the FARMFLORA birth cohort, compared to 11 of 37 (30%) control children living in the same rural area (125), which is in line with previous studies (29, 34, 35). In the present thesis, we aimed to investigate whether the dietary habits of farming families may be part of the protection conferred by growing up on farms. Differences in the very early diet of farm and control children, including maternal diet during pregnancy and lactation as well as the fatty acid composition of breast milk and its relation to the fatty acid composition of infant sera, were evaluated. We also analyzed differences in the timing of introducing complementary foods, and the children's diet at the age of one year. The same variables were then related to allergy development at three years of age.

DIETARY PATTERNS OF FARMERS AND CONTROLS

We found that both the farming mothers and their children consumed more full-fat dairy products and saturated fatty acids compared to the control families. These findings may well be expected in dairy farmers, and were in line with previous studies (11-14, 42). However, to the best of our knowledge, the diet has not previously been evaluated at such an early age as in our study, and in no other study has the fatty acid composition of both breast milk and children's sera been evaluated in the same subjects. We found that higher intake of full-fat dairy and saturated fatty acids in farming mothers coincided with higher proportions of saturated fatty acids in the breast milk as well as a higher intake of full-fat dairy and saturated fats in the children as measured at one year of age. Concordantly, the intake of margarine and vegetable oils was lower in the farming mothers, as was the proportion of the n-3 PUFA alpha-linolenic acid, and the n-6 PUFA linoleic acid was lower in their breast milk. Tendencies toward a lower margarine intake in the one-year-old farm children were consistent with the lower margarine intake in the farming mothers. However, the only difference found in the sera of farming and control children were higher proportions of the monounsaturated fatty acid 18:1 n-7, of which the proportions did not correlate to the proportions in breast-milk. Unfortunately, we did not analyze saturated fatty acids with a shorter carbon chain than 18. Hence, we cannot rule out that there might be differences in serum in other saturated fatty acids than those we measured. The saturated fatty acid 14:0 has been proposed to be a good marker for intake of dairy product (128), in contrast to for example 18:0, which may be converted to 18:1 in the body. For the analysis of cord sera, a larger range of fatty acids was measured compared to 4 month sera, including 14:0. Indeed, we found that 14:0 correlated with the mothers' intake of whole milk during pregnancy (paper III). Interestingly, the only fatty acid that differed in cord sera between farm and control children was arachidonic acid. However, no correlations were found between this fatty acid and the mothers' diet during pregnancy.

No correlations of full-fat dairy products, butter, margarine or fish were found between the diet of the children in the control group and their mothers' diet. One explanation may be the relatively low and probably irregular consumption of these products. However, margarines were consumed to a higher extent in control families than in farming families; still a correlation between the children's and the mothers' intake was only found in the farming group. The reason for this is unclear.

Do dietary factors explain the allergy protection conferred by the farming environment?

An inverse association between allergy and the consumption of butter (14, 16, 17, 68, 69) and full-fat dairy (16, 75) has been observed in a number of studies. However, we could not link the higher consumption of butter and full-fat dairy products in the farming families to the lower prevalence of allergy in the children. Consumption of unpasteurized farm milk has been associated with allergy protection in several studies (36, 38, 39, 82) and has, together with regular contact with livestock and fodder, been proposed to be an explanatory factor for the allergy protection in the farming environment (34, 38).

The effect of neither pasteurized farm milk nor unpasteurized farm milk on allergy development could be evaluated in this thesis, since only farming families consumed such milk and there was only one allergic subject in the farming group at the age of three years. On the other hand, we found that the lower intake of margarines and vegetable oils in the farming mothers, as well as a lower intake of margarines in the children at one year of age, were weakly associated with a lower risk of allergy development. Since only one farm child was allergic, we evaluated the association between margarine and allergy in both farm and control children, as well as in control children only. The mothers of subsequently allergic children still consumed significantly more margarines and oils during lactation, and borderline significantly more margarines and oils during pregnancy, as compared to mothers of healthy children. Using logistic regression, the intake also during pregnancy was significantly associated with allergy in the control group, as was the children's intake at one year of age. Adjusting for potential covariates did not modify the associations notably. Not surprisingly, the intake of margarines correlated among the children and their mothers. In addition, consumption of margarines in the family correlated negatively with the proportions of EPA in the infants' sera at four months of age, although only significantly for the mothers' intake during pregnancy. Since the serum proportions of EPA were markedly lower in subsequently allergic children, this negative correlation may account for the association between margarines and allergy. However, the strongest negative correlation was found between serum EPA and maternal intake of margarines during pregnancy, while, on the other hand, the strongest association with allergy in their children was found for the mothers' intake of both margarine and oils. In univariable analysis, the children's allergy was more strongly related to the mothers' intake of margarines and oils during lactation, which correlated only weakly and insignificantly with EPA. Hence, the intake of margarine seems, at least partly, to exert an independent effect on the development of allergy in this cohort.

Positive associations between margarine intake and allergy have been shown previously (18, 79, 89). An increased intake of margarines and oils, which are good sources of the n-6 PUFA linoleic acid, was hypothesized in 1997 by Black and Sharpe to explain the allergy increase (20). Linoleic acid is a precursor of arachidonic acid and according to this hypothesis it is converted to prostaglandin E_2, which, in turn, acts on T-lymphocytes to reduce interferon-ɣ but does not affect the formation of IL-4. Interleukin-4 stimulates the synthesis of IgE, while interferon-ɣ has the opposite effect. Hence, Black and Sharpe proposed that the increase in linoleic acid intake may increase IL-4 and decrease interferon-ɣ and thereby promote sensitization. However, no difference was found between the healthy and allergic mothers in our study in their intake of linoleic acid, and the intake of linoleic acid correlated neither with the intake of margarines alone nor with the intake of margarines and oils together.

To the best of our knowledge, an association between margarine and allergy has not been found in any other study, in which the relationship between farmers' diet and allergy have been evaluated, which makes this a novel finding as one potential contributory factor to the low prevalence of

atopic diseases in farmers' children. However, the relationship between maternal consumption of margarines and oils and allergy development was significant at a level $P > 0.01$. Due to the risk of our finding being a chance finding, as a result of multiple comparisons, these results must be interpreted with caution.

FISH INTAKE AND ALLERGY DEVELOPMENT

In our study, we found a vague line of higher intakes of oily fish or seafood in the healthy mothers and their children as well as a correlation between higher proportions of long-chain n-3 PUFAs in both breast milk and infant sera, and protection against allergy development. The EPA proportions in the infants' sera, both at birth and at four months of age, were associated with a lower risk of allergy development. This association was also robust after adjusting for potential covariates, especially regarding sera at four months of age. The proportions of EPA measured at both time points correlated with the mothers' intake of oily fish. The proportions of EPA and n-3 PUFA in infant sera at four months of age correlated with the corresponding fatty acids in breast-milk. Higher proportions of the n-3 long-chain PUFA DHA in breast milk were associated with lower odds of developing allergy. The breast milk proportions of the long-chain n-3 PUFAs, in turn, correlated with the mothers' intake of oily fish during pregnancy and lactation. In line with these results, healthy children consumed more seafood and had fish introduced earlier into their diet as compared to children who developed allergy, although these associations lost significance when the analysis was restricted to control children only. This could either be explained by a lower power resulting from the smaller study sample or a slightly similar trend of a higher/earlier fish intake in farm children, who developed allergy to a lower extent than controls, accounting for part of the association observed between being healthy and allergy protection. The farm children consumed slightly more oily fish at one year of age than controls. However, there is no rationale behind a consistent higher fish intake in inland dairy farmers compared to their non-farming neighbors, and, hence, it is not very likely that a higher fish intake in farmers accounts for the allergy protection from growing up on a farm. Indeed, no difference in the consumption of oily fish, and a lower intake of lean fish, was observed in six-year-old farm children compared to controls, as reported by Remes and coworkers (12).

Although consumption of fish may not consistently characterize farmers' diets, a diet rich in fish has been linked to allergy protection in numerous studies; an association has been observed for the mothers intake during pregnancy (18, 21, 78, 91, 95-97), and the early introduction of fish to the diet of the child itself (47, 48, 54-60) and intake of fish later on in their regular diet (22, 57, 90, 98-100). The protective effect of fish has been ascribed to its high content of long-chain n-3 PUFAs in many studies (22, 97, 102, 103). These findings are in line with the results of this thesis of the markedly lower proportions of EPA in the sera of subsequently allergic as compared to healthy children. However, a stronger protective effect of fish as a whole, and not only its n-3 PUFA content, is indicated by other studies (22, 54). Consequently, the n-3 proportions in the healthy infants' sera found in our study may act as a proxy for a pattern of a higher fish intake mothers both during pregnancy and during lactation, as indicated by the strong correlations between long-chain n-3 PUFAs in serum and breast milk and maternal intake of oily fish.

When evaluating our results regarding allergy and fish and margarine consumption in relation to the existing literature, it must be kept in mind that it is difficult to obtain a full picture. Both margarines and fish are likely to have been evaluated in numerous studies, although incorporated in the evaluation of the diet as a whole and not as specific primary outcomes. In cases of no relationships, these results are often not emphasized, or not even mentioned that they have been measured, to leave room for significant associations between other food items and allergy. However, the associations that have been found to be significant between allergy and margarine,

and especially between allergy and fish, generally point in the same direction. Nevertheless, this must be interpreted with caution due to the difficulties of proper systematic reviews and meta-analyses.

INTRODUCTION OF COMPLEMENTARY FOODS AND ALLERGY DEVELOPMENT

We observed a pattern of an earlier introduction of most food groups in the healthy children in our cohort, as compared to the subsequently allergic children, which is in line with a growing number of studies (47-50, 129). Postponed introduction of fish has repeatedly been observed to be associated with a higher risk of developing allergy (47, 48, 54-60). Concordantly, we found a pattern of later introduction of fish in subsequently allergic, as compared to healthy, children. By exploring this relationship more closely, we found that a significantly higher percentage of allergic children had fish introduced after ten months of age. This was also the case for the introduction of eggs, which had been introduced after ten months of age in a higher number of allergic subjects. The differences observed for fish between the healthy and allergic children did not reach significance when the analysis was confined to control subjects, which may be a result of the small sample size, and hence the low power. We do not believe that our results were affected by reverse causation, since we asked the families in retrospect whether they deliberately postponed the introduction of certain foods due to a family history of atopy or early signs of allergy. Sixty-two percent responded to this question, and all responded negatively, despite a family history of allergy in approximately half of the responding families.

Due to the low number of subjects, we were not able to investigate specific phenotypes of atopy, which may have masked significant associations to only one or a few phenotypes. This might not least be the case for food allergies, since two recent, very large and well conducted randomized controlled trials have shown that early introduction of peanuts (64, 65) and egg (65) were associated with a distinctly lower risk of developing allergies towards these specific foods. Furthermore, any food allergy was lower in those children that were randomized to the intervention group of early introduction (three months) of six foods that are commonly found to be the target of food allergies, provided that they received the amounts of food specified in the protocol (per protocol analysis) (65).

CURRENT RECOMMENDATIONS

The general population, as well as pregnant and lactating mothers and young children, are advised to exchange animal fats for vegetable fats, including margarines and oils, according to current recommendations (118). The need for a proper evaluation of the effect of margarine on allergy development is emphasized by our results, which follow a series of previous studies (14, 15, 17-19, 68, 78, 79, 81, 84-89). To date, no randomized controlled trials have been conducted on this matter, to the best of our knowledge. Moreover, exclusive breastfeeding is recommended up to the age of six months, and complementary foods are not to be introduced before the age of four to six months (46). In current Swedish national guidelines, a proper introduction of complementary foods is clearly stated not to commence before the age of six months. In light of the emerging number of studies showing benefits of introduction foods even earlier than six months of age (47, 48, 65), current recommendations may be contra-productive regarding the development of tolerance to a broad diversity of foods and a proper stimulation of the immune system. Feeding the infant tiny amounts of foods and exposing the child to miniscule amounts of potentially allergenic proteins may increase the risk of sensitization in the child and, hence, the risk of allergy development (25, 28), since quite large doses appear to be needed in order to develop oral tolerance (25, 28, 65).

STRENGTHS AND WEAKNESSES

The prospective design and the allergy diagnoses assessed clinically by pediatricians are two great strengths of the FARMFLORA birth cohort and the studies included in this thesis. Clinical diagnoses of atopic diseases that are not parent- or self-reported are found only in few other studies, while most observational studies include parental/self-reported symptoms of allergy, of which some are reported to be diagnosed by a physician. Even the controlled intervention study by Dotterud and coworkers included parents' reports of allergy (21). Naturally, most studies of the introduction of complementary foods and maternal intake during pregnancy and lactation, and subsequent allergy in the child have prospective design, although there are exceptions (78, 97). However, the majority of observational studies in which the relationship between farming or dietary exposures and allergy development is evaluated are cross-sectional. Another strength of the FARMFLORA cohort is that the children from the control group lives in the same rural area as the farm group, which eliminates confounding factors conferred by an urban environment.

Regarding the dietary assessment during pregnancy, one may speculate over how well a mother that recently gave birth to her child remembers her pregnancy diet. At the same time, the time of pregnancy may be one period in a woman's life during which she is very well aware of what she eats, not least for a number of restrictions that are advocated for pregnant women. The dietary data in this thesis are strengthened by the coherence of the results obtained using different approaches to evaluate the diet, including an FFQ during pregnancy for a long-term dietary picture and a detailed 48 hour assessment during lactation - both showing the same patterns at the group level - as well as a fatty acid composition of breast milk that corroborates the dietary data.

The convenient sample size of 65 subjects in the FARMFLORA birth cohort has permitted us to follow the children closely with detailed assessments of numerous parameters, at several time points, including the diets of the mothers and the children and clinical allergy diagnoses, as well as strictly verify the eligibility of the subjects in the farming group. As a drawback, the small sample size also results in both a low power, which increases the risk of type II errors and difficulties in a proper adjustment for confounders. However, despite the size of the cohort, we were able to detect several significant differences that we evaluated in separate logistic regression models by including one potential covariate at a time. By this means, we could not observe the sum of the effects of each covariate on the effect of the exposure, although we were able to obtain indications of the robustness of our results. Moreover, a large number of parameters were evaluated in relation to farm residence and allergy development, which increases the risk of type I errors. However, we chose an explorative approach to make the best use of our detailed measurements, although we interpreted our results with caution and did not emphasize weak results not supported by previous literature.

Despite our small sample size, a substantial number of samples were lacking in some parts of our sampling. Most notably, this was the case for the samples of breast milk that were obtained from only five of 11 allergic children. Three missing samples were a result of the mothers having stopped breastfeeding, while the remaining three missing samples were merely a result of the families not having provided samples. Despite the fact that we had breast milk samples from only five allergic children, we observed higher proportions of DHA in the breast milk from mothers of healthy children. The same marked difference remained when farm children were excluded from the analysis, although the significance disappeared. The weak *P*-value is very likely a result of the low power resulting from the few breast milk samples in the allergic group, and may well have been stronger in a larger subset of subjects, not least in light of the strong significances observed for the other long-chain n-3 PUFA in the children's sera.

KEY FINDINGS

- Mothers of farm children consumed more butter and full-fat dairy products during pregnancy and lactation as compared to mothers of control children, who consumed more margarine and vegetable oils. These differences were reflected in breast milk as higher proportions of saturated fat and lower proportions of polyunsaturated fat in the farming mothers. The mothers' consumption of margarine and oils during pregnancy and lactation, as well as the children's intake of margarines at one year of age, were weakly associated with allergy development in the children at three years of age. Breast milk proportions of the long-chain n-3 PUFA DHA tended to be inversely related to allergy development, although no significant differences were found in the breast milk of farm and control mothers.

- Despite differences in breast milk, the composition of fatty acids in sera of four-month-old farm and control children was similar, except for lower proportions of the n-7 fatty acid 18:1 in farm children. Healthy children had distinctively higher proportions of the long-chain n-3 PUFA EPA than subsequently allergic children. Serum EPA proportions were unrelated to farming status. The proportions of long-chain n-3 PUFAs in the infants' sera correlated with the proportions of these fatty acids in breast milk, which, in turn, correlated with maternal fish intake during both pregnancy and lactation.

- The introduction of complementary foods was similar in farming and non-farming families, apart from nuts that were introduced earlier in the farm children's diet. When healthy and subsequently allergic children were compared, a pattern of earlier introduction was observed among the healthy children. A significantly higher rate of healthy children was exclusively breastfed during the first three months or life, although this was only significantly higher up to one month when the analysis was confined to control children. When adjusted for exclusive breastfeeding, earlier introduction of flour was significantly related to a reduced risk of developing allergy. A higher rate of allergic children had eggs and tended to have fish introduced after ten months of age.

- At one year of age, the diet of the farm children contained more full-fat dairy and tended to contain more butter and less margarine than controls, which resembled the dietary pattern of the farming mothers during pregnancy and lactation. Healthy children consumed more seafood than allergic children, although the significance was lost in the case of control children only. However, healthy control children consumed significantly less pork and zinc than subsequently allergic control children.

48

CONCLUSIONS

- A similar pattern was found in the farm children's diet at one year of age and the diet of their mothers during pregnancy and lactation, including a higher intake of butter and full-fat dairy and a lower intake of margarines. The intake of margarines in the diet of both the mothers and the children was weakly associated with allergy in the children at three years of age. Hence, a low margarine intake may partly explain the low prevalence of allergy in farmers' children.

- The fatty acid pattern in the breast milk of farming mothers reflected their higher intake of saturated fat and lower intake of margarines and oils compared to control mothers. This pattern was not found in the farm infant's sera, however, which basically contained similar proportions of fatty acids as the sera of control infants, and the allergy protective 'farm effect' could not be linked to a distinct fatty acid pattern in sera.

- Although not related to farm residence, high serum proportions of the long-chain n-3 PUFA EPA that reflected breast milk proportions of long-chain n-3 PUFAs and maternal fish intake were associated with protection against allergy development. Concordantly, the children's intake of seafood at one year of age and having had fish introduced to the diet at ten months of age, compared to after this age, were weakly associated with allergy protection. Hence, including fish in the diet of infants and their mothers may offer protection against allergy development.

- A pattern of earlier introduction of a number of food groups was found in healthy children, as compared to subsequently allergic children, although exclusive breastfeeding up to three months of age was more common among the healthy children. The introduction of complementary foods did not differ notably between farm and control children and could not be concluded to account for part of the 'farm protection'.

- Our results concerning a potentially allergy protective effect of a diet rich in fish are in line with current dietary recommendations, including an increased consumption of fish in general. However, our results regarding an increased risk of margarine consumption warrant more research to investigate a causal relationship, especially in the lights that an increased consumption of margarines instead of butter is currently recommended to increase the intake of PUFAs. In addition, our results may challenge the national recommendations regarding exclusive breastfeeding up to the age of six months. Possible benefits may be obtained by a more tolerant, and even encouraging, approach in terms of introducing complementary foods already at the age of four months.

FUTURE PERSPECTIVES

We observed positive associations between allergy at three years of age and margarine consumption in the mothers during pregnancy and lactation and in the children at one year of age. Consumption of margarines instead of butter is advocated in the current national guidelines in order to increase the intake of polyunsaturated fats and decrease the intake of saturated fats. Large prospective studies, in which potential confounding factors are properly controlled for, are needed to strengthen our findings, as are randomized controlled trials to evaluate a causal relationship between margarine intake and allergy development.

Proportions of EPA in infant sera at birth and at four months of age were strongly inversely associated with allergy at three years of age. We have recently obtained allergy diagnoses at the median age of nine years of the children. Still at this age, we found an inverse association between allergy and EPA proportions in sera both at four months of age and at birth. The association between prenatal and early post-natal exposures and allergy development is in prospective studies often evaluated in relation to outcome measures in early childhood. Evaluating the relationship with allergy up to the age of nine years is a strength, and the preliminary findings based on our data among nine-year-olds should be evaluated further.

In the large, randomized controlled studies LEAP and EAT, early introduction of potentially allergenic foods was observed to decrease the risk of food allergy. Our results point to early introduction of certain foods possibly also being protective against other types of allergies. In the EAT study, the effect of early food introduction on other types of allergies is presently investigated. Forthcoming results may, together with the previous results of not least the EAT and LEAP studies, induce changes in our current national recommendations of exclusive breastfeeding up to six months of age.

Future studies are needed to explain the mechanisms behind the associations between allergy and margarines, EPA and fish and the introduction of complementary foods

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REFERENCES

1. Asher M, Montefort S, Bjorksten B, Lai C, Strachan D, Weiland S, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. The Lancet. 2007;368:733 - 43.
2. Eder W, Ege MJ, von Mutius E. The asthma epidemic. The New England Journal of Medicine. 2006;355:2226 - 35.
3. Miljöhälsorapport 2013. Stockholm, Sweden: Institute of Environmental Medicine; 2013. Report No.: 978-91-637-3031-3.
4. Mölne J, Wold A. Inflammation. Stockholm: Liber; 2007.
5. Strachan DP. Hay fever, hygiene, and household size. BMJ. 1989;299(6710):1259-60.
6. Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? Allergy. 1998;53(46 Suppl):20-5.
7. Martricardi P, Ronchetti R. Are infections protecting from atopy? Curr Opin Allergy Clin Immunol. 2001;1(5):413 - 9.
8. Wang M, Karlsson C, Olsson C, Adlerberth I, Wold AE, Strachan DP, et al. Reduced diversity in the early fecal microbiota of infants with atopic eczema. Journal of Allergy and Clinical Immunology. 2008;121(1):129-34.
9. Blackley CH. Experimental Researches on the Causes and Nature of Catarrhus Aestivus (Hay-Fever or Hay-Asthma). 1873.
10. von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. Nature reviews. 2010;10(12):861-8.
11. Michels KB, Waser M, Ary E, Rosenlund H, Von Mutius E, Riedler J, et al. Validation of a questionnaire to assess dietary habits among 5-13-year old school children of farmers and anthroposophic families. J Nutr Environ Med. 2008;17(3):157-68.
12. Remes ST, Iivanainen K, Koskela H, Pekkanen J. Which factors explain the lower prevalence of atopy amongst farmers' children? Clin Exp Allergy. 2003;33(4):427-34.
13. Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Bohm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy. 2000;30(2):187-93.
14. Waser M, Michels KB, Bieli C, Flöistrup H, Pershagen G, Von Mutius E, et al. Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe. Clin Exp Allergy. 2007;37(5):661-70.
15. Farchi S, Forastiere F, Agabiti N, Corbo G, Pistelli R, Fortes C, et al. Dietary factors associated with wheezing and allergic rhinitis in children. Eur Respir J. 2003;22(5):772-80.
16. Wijga AH, Smit HA, Kerkhof M, de Jongste JC, Gerritsen J, Neijens HJ, et al. Association of consumption of products containing milk fat with reduced asthma risk in pre-school children: the PIAMA birth cohort study. Thorax. 2003;58(7):567-72.
17. von Mutius E, Weiland SK, Fritzsch C, Duhme H, Keil U. Increasing prevalence of hay fever and atopy among children in Leipzig, East Germany. Lancet. 1998;351(9106):862-6.
18. Sausenthaler S, Koletzko S, Schaaf B, Lehmann I, Borte M, Herbarth O, et al. Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 y of age. Am J Clin Nutr. 2007;85(2):530-7.
19. Sausenthaler S, Kompauer I, Borte M, Herbarth O, Schaaf B, Berg A, et al. Margarine and butter consumption, eczema and allergic sensitization in children. The LISA birth cohort study. Pediatr Allergy Immunol. 2006;17(2):85-93.
20. Black PN, Sharpe S. Dietary fat and asthma: is there a connection? Eur Respir J. 1997;10(1):6-12.
21. Dotterud CK, Storrø O, Simpson MR, Johnsen R, Øien T. The impact of pre- and postnatal exposures on allergy related diseases in childhood: a controlled multicentre intervention study in primary health care. BMC Public Health. 2013;13:123.
22. Øien T, Storrø O, Johnsen R. Do early intake of fish and fish oil protect against eczema and doctor-diagnosed asthma at 2 years of age? A cohort study. Journal of Epidemiology and Community Health. 2010;64(2):124-9.
23. Lack G. Epidemiologic risks for food allergy. Journal of Allergy and Clinical Immunology. 2008;121(6):1331-6.

24. Lack G. Update on risk factors for food allergy. Journal of Allergy and Clinical Immunology. 2012;129(5):1187-97.

25. Wells HG, Osborne TB. The biological reactions of the vegetable proteins i. anaphylaxis. J Infect Dis. 1911;8(1):66-124.

26. Chase MW. Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine (New York, NY). 1946;61:257-9.

27. Husby S, Mestecky J, Moldoveanu Z, Holland S, Elson CO. Oral tolerance in humans: T cell but not B cell tolerance after antigen feeding. Journal of Immunology. 1994;152(9):4663-70.

28. Lamont AG, Mowat McI A, Parrott DMV. Priming of systemic and local delayed-type hypersensitivity responses by feeding low doses of ovalbumin to mice. Immunology. 1989;66(4):595-9.

29. Braun-Fahrlander C, Gassner M, Grize L, Neu U, Sennhauser FH, Varonier HS, et al. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution. Clin Exp Allergy. 1999;29(1):28-34.

30. Aberg N. Asthma and allergic rhinitis in Swedish conscripts. Clin Exp Allergy. 1989;19:59-63.

31. Iversen M, Pedersen B. The prevalence of allergy in Danish farmers. Allergy. 1990;45:347-53.

32. Rautalahti M, Terho EO, Vohlonen I, Husman K. Atopic sensitization of dairy farmers to work-related and common allergens. Eur J Respir Dis. 1987;71(Suppl. 152):155-64.

33. Alfvén T, Braun-FahrlÃ¤nder C, Brunekreef B, Von Mutius E, Riedler J, Scheynius A, et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle - The PARSIFAL study. Allergy: European Journal of Allergy and Clinical Immunology. 2006;61(4):414-21.

34. Ege MJ, Frei R, Bieli C, Schram-Bijkerk D, Waser M, Benz MR, et al. Not all farming environments protect against the development of asthma and wheeze in children. Journal of Allergy and Clinical Immunology. 2007;119(5):1140-7.

35. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin Exp Allergy. 2000;30(2):194-200.

36. Douwes J, Cheng S, Travier N, Cohet C, Niesink A, McKenzie J, et al. Farm exposure in utero may protect against asthma, hay fever and eczema. European Respiratory Journal. 2008;32(3):603-11.

37. Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Ãœblagger E, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. Journal of Allergy and Clinical Immunology. 2006;117(4):817-23.

38. Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? J Allergy Clin Immunol. 2006;117(6):1374-81.

39. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet. 2001;358(9288):1129-33.

40. Merchant JA, Naleway AL, Svendsen ER, Kelly KM, Burmeister LF, Stromquist AM, et al. Asthma and farm exposures in a cohort of rural Iowa children. Environ Health Perspect. 2005;113(3):350-6.

41. Midodzi WK, Rowe BH, Majaesic CM, Senthilselvan A. Reduced risk of physician-diagnosed asthma among children dwelling in a farming environment. Respirology. 2007;12(5):692-9.

42. Lande B, Almås R. Kostholdsvaner i et utvalg av norske bønder. Scandinavian Journal of Nutrition. 1992;37:23-7.

43. Fergusson DM, Horwood LJ. Early solid food diet and eczema in childhood: a 10-year longitudinal study. Pediatric Allergy and Immunology. 1994;5(5 S):44-7.

44. Kajosaari M. Atopy prevention in childhood: the role of diet Prospective 5-year follow-up of high-risk infants with six months exclusive breastfeeding and solid food elimination. Pediatric Allergy and Immunology. 1994;5(5 S):26-8.

45. American Academy of Pediatrics. Committee on Nutrition. Hypoallergenic infant formulas. Pediatrics. 2000;106(2 Pt 1):346-9.

46. Greer FR, Sicherer SH, Burks AW. Effects of Early Nutritional Interventions on the Development of Atopic Disease in Infants and Children: The Role of Maternal Dietary Restriction, Breastfeeding,

Timing of Introduction of Complementary Foods, and Hydrolyzed Formulas. Pediatrics. 2008;121(1):183-91.

47. Nwaru BI, Takkinen HM, Niemelä O, Kaila M, Erkkola M, Ahonen S, et al. Timing of infant feeding in relation to childhood asthma and allergic diseases. Journal of Allergy and Clinical Immunology. 2013;131(1):78-86.

48. Nwaru BI, Takkinen HM, Niemelä O, Kaila M, Erkkola M, Ahonen S, et al. Introduction of complementary foods in infancy and atopic sensitization at the age of 5 years: Timing and food diversity in a Finnish birth cohort. Allergy: European Journal of Allergy and Clinical Immunology. 2013;68(4):507-16.

49. Sariachvili M, Droste J, Dom S, Wieringa M, Hagendorens M, Stevens W, et al. Early exposure to solid foods and the development of eczema in children up to 4 years of age. Pediatric Allergy and Immunology. 2010;21(1 PART I):74-81.

50. Turati F, Bertuccio P, Galeone C, Pelucchi C, Naldi L, Bach JF, et al. Early weaning is beneficial to prevent atopic dermatitis occurrence in young children. Allergy: European Journal of Allergy and Clinical Immunology. 2016;71(6):878-88.

51. Nwaru BI, Takkinen HM, Kaila M, Erkkola M, Ahonen S, Pekkanen J, et al. Food diversity in infancy and the risk of childhood asthma and allergies. Journal of Allergy and Clinical Immunology. 2014;133(4):1084-91.

52. Roduit C, Frei R, Depner M, Schaub B, Loss G, Genuneit J, et al. Increased food diversity in the first year of life is inversely associated with allergic diseases. Journal of Allergy and Clinical Immunology. 2014;133(4):1056-64.e7.

53. Roduit C, Frei R, Loss G, Büchele G, Weber J, Depner M, et al. Development of atopic dermatitis according to age of onset and association with early-life exposures. Journal of Allergy and Clinical Immunology. 2012;130(1):130-6.e5.

54. Alm B, Aberg N, Erdes L, Mollborg P, Pettersson R, Norvenius SG, et al. Early introduction of fish decreases the risk of eczema in infants. Archives of disease in childhood. 2009;94(1):11-5.

55. Hesselmar B, Saalman R, Rudin A, Adlerberth I, Wold AE. Early fish introduction is associated with less eczema, but not sensitization, in infants. Acta Paediatr Int J Paediatr. 2010;99(12):1861-7.

56. Kiefte-de Jong JC, De Vries JH, Franco OH, Jaddoe VWV, Hofman A, Raat H, et al. Fish consumption in infancy and asthma-like symptoms at preschool age. Pediatrics. 2012;130(6):1060-8.

57. Kull I, Bergström A, Lilja G, Pershagen G, Wickman M. Fish consumption during the first year of life and development of allergic diseases during childhood. Allergy. 2006;61(8):1009-15.

58. Lumia M, Takkinen HM, Luukkainen P, Kaila M, Lehtinen-Jacks S, Nwaru BI, et al. Food consumption and risk of childhood asthma. Pediatr Allergy Immunol. 2015;26(8):789-96.

59. Nafstad P, Nystad W, Magnus P, Jaakkola JJ. Asthma and allergic rhinitis at 4 years of age in relation to fish consumption in infancy. J Asthma. 2003;40(4):343-8.

60. Goksör E, Alm B, Thengilsdottir H, Pettersson R, Åberg N, Wennergren G. Preschool wheeze - Impact of early fish introduction and neonatal antibiotics. Acta Paediatr Int J Paediatr. 2011;100(12):1561-6.

61. McKean M, Caughey AB, Leong RE, Wong A, Cabana MD. The timing of infant food introduction in families with a history of atopy. Clin Pediatr. 2015;54(8):745-51.

62. Tromp IIM, Briedé S, Kiefte-De Jong JC, Renders CM, Jaddoe VWV, Franco OH, et al. Factors associated with the timing of introduction of complementary feeding: The Generation R Study. European Journal of Clinical Nutrition. 2013;67(6):625-30.

63. Ribeiro CC, Leite Speridião PDG, Morais MBD. Knowledge and practice of physicians and nutritionists regarding the prevention of food allergy. Clin Nutr. 2013;32(4):624-9.

64. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. New England Journal of Medicine. 2015;372(9):803-13.

65. Perkin MR, Logan K, Tseng A, Raji B, Ayis S, Peacock J, et al. Randomized trial of introduction of allergenic foods in breast-fed infants. New England Journal of Medicine. 2016;374(18):1733-43.

66. Hanson DG, Vaz NM, Maia LCS, Hornbrook MM, Lynch JM, Roy CA. Inhibition of specific immune responses by feeding protein antigens. Int Arch Allergy Immunol. 1977;55(1-6):526-32.

67. Vreeburg KJJ, de Groot K, Von Blomberg M, Scheper RJ. Induction of Immunological Tolerance by Oral Administration of Nickel and Chromium. J Dent Res. 1984;63(2):124-8.

68. Dunder T, Kuikka L, Turtinen J, Rasanen L, Uhari M. Diet, serum fatty acids, and atopic diseases in childhood. Allergy. 2001;56(5):425-8.

69. Johansson S, Wold AE, Sandberg AS. Low breast milk levels of long-chain n-3 fatty acids in allergic women, despite frequent fish intake. Clin Exp Allergy. 2011;41(4):505-15.

70. Suarez-Varela MM, Alvarez LG, Kogan MD, Ferreira JC, Martinez Gimeno A, Aguinaga Ontoso I, et al. Diet and prevalence of atopic eczema in 6 to 7-year-old schoolchildren in Spain: ISAAC phase III. J Investig Allergol Clin Immunol. 2010;20(6):469-75.

71. Kummeling I, Thijs C, Huber M, van de Vijver LPL, Snijders BEP, Penders J, et al. Consumption of organic foods and risk of atopic disease during the first 2 years of life in the Netherlands. British Journal of Nutrition. 2008;99(3):598-605.

72. Nwaru BI, Erkkola M, Ahonen S, Kaila M, Lumia M, Prasad M, et al. Maternal diet during lactation and allergic sensitization in the offspring at age of 5. Pediatric Allergy and Immunology. 2011;22(3):334-41.

73. Snijders BEP, Thijs C, van Ree R, van den Brandt PA. Age at First Introduction of Cow Milk Products and Other Food Products in Relation to Infant Atopic Manifestations in the First 2 Years of Life: The KOALA Birth Cohort Study. Pediatrics. 2008;122(1):e115-e22.

74. Wijga A, Houwelingen AC, Smit HA, Kerkhof M, Vos AP, Neijens HJ, et al. Fatty acids in breast milk of allergic and non-allergic mothers: The PIAMA birth cohort study. Pediatr Allergy Immunol. 2003;14(3):156-62.

75. Woods RK, Walters EH, Raven JM, Wolfe R, Ireland PD, Thien FCK, et al. Food and nutrient intakes and asthma risk in young adults. American Journal of Clinical Nutrition. 2003;78(3):414-21.

76. Waser M. Children who regularly drink milk directly from the farm, rarely develop allergic diseases. Kinder, die regelmäßig milch direkt vom bauernhof trinken, entwickeln seltener allergische erkrankungen. 2007;67(9):367-8.

77. Lumia M, Luukkainen P, Tapanainen H, Kaila M, Erkkola M, Uusitalo L, et al. Dietary fatty acid composition during pregnancy and the risk of asthma in the offspring. Pediatric Allergy and Immunology. 2011;22(8):827-35.

78. Calvani M, Alessandri C, Sopo SM, Panetta V, Pingitore G, Tripodi S, et al. Consumption of fish, butter and margarine during pregnancy and development of allergic sensitizations in the offspring: role of maternal atopy. Pediatr Allergy Immunol. 2006;17(2):94-102.

79. Lumia M, Luukkainen P, Kaila M, Tapanainen H, Takkinen HM, Prasad M, et al. Maternal dietary fat and fatty acid intake during lactation and the risk of asthma in the offspring. Acta Paediatr Int J Paediatr. 2012;101(8):e337-e43.

80. Nwaru BI, Erkkola M, Lumia M, Kronberg-Kippilä C, Ahonen S, Kaila M, et al. Maternal intake of fatty acids during pregnancy and allergies in the offspring. The British journal of nutrition. 2012;108(04):720-32.

81. Takaoka M, Norback D. Diet among Japanese female university students and asthmatic symptoms, infections, pollen and furry pet allergy. Respir Med. 2008;102(7):1045-54.

82. Ege MJ, Herzum I, Büchele G, Krauss-Etschmann S, Lauener RP, Roponen M, et al. Prenatal exposure to a farm environment modifies atopic sensitization at birth. Journal of Allergy and Clinical Immunology. 2008;122(2).

83. Loss G, Depner M, Ulfman LH, Van Neerven RJJ, Hose AJ, Genuneit J, et al. Consumption of unprocessed cow's milk protects infants from common respiratory infections. Journal of Allergy and Clinical Immunology. 2015;135(1):56-62.

84. Akcay A, Tamay Z, Hocaoglu AB, Ergin A, Guler N. Risk factors affecting asthma prevalence in adolescents living in Istanbul, Turkey. Allergol Immunopathol. 2014;42(5):449-58.

85. Bolte G, Frye C, Hoelscher B, Meyer I, Wjst M, Heinrich J. Margarine Consumption and Allergy in Children. Am J Respir Crit Care Med. 2001;163(1):277-9.

86. Bolte G, Winkler G, Holscher B, Thefeld W, Weiland SK, Heinrich J. Margarine consumption, asthma, and allergy in young adults: results of the German National Health Survey 1998. Ann Epidemiol. 2005;15(3):207-13.

87. Chatzi L, Apostolaki G, Bibakis I, Skypala I, Bibaki-Liakou V, Tzanakis N, et al. Protective effect of fruits, vegetables and the Mediterranean diet on asthma and allergies among children in Crete. Thorax. 2007;62(8):677-83.

88. Nagel G, Linseisen J. Dietary intake of fatty acids, antioxidants and selected food groups and asthma in adults'. Eur J Clin Nutr. 2005;59(1):8-15.

89. Sausenthaler S, Koletzko B, Heinrich J. Dietary fat intake and allergic diseases. Curr Nutr Food Sci 2006;2(4):351-9.

90. Chatzi L, Torrent M, Romieu I, Garcia-Esteban R, Ferrer C, Vioque J, et al. Diet, wheeze, and atopy in school children in Menorca, Spain. Pediatr Allergy Immunol. 2007;18(6):480-5.

91. Willers SM, Devereux G, Craig LCA, McNeill G, Wijga AH, Abou El-Magd W, et al. Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms in 5-year-old children. Thorax. 2007;62(9):772-8.

92. Goksör E, Alm B, Pettersson R, Möllborg P, Erdes L, Åberg N, et al. Early fish introduction and neonatal antibiotics affect the risk of asthma into school age. Pediatric Allergy and Immunology. 2013;24(4):339-44.

93. Nwaru BI, Erkkola M, Ahonen S, Kaila M, Haapala AM, Kronberg-Kippila C, et al. Age at the introduction of solid foods during the first year and allergic sensitization at age 5 years. Pediatrics. 2010;125(1):50-9.

94. Virtanen SM, Kaila M, Pekkanen J, Kenward MG, Uusitalo U, Pietinen P, et al. Early introduction of oats associated with decreased risk of persistent asthma and early introduction of fish with decreased risk of allergic rhinitis. British Journal of Nutrition. 2010;103(02):266-73.

95. Jedrychowski W, Perera F, Maugeri U, Mrozek-Budzyn D, Miller RL, Flak E, et al. Effects of prenatal and perinatal exposure to fine air pollutants and maternal fish consumption on the occurrence of infantile eczema. Int Arch Allergy Immunol. 2011;155(3):275-81.

96. Romieu I, Torrent M, Garcia-Esteban R, Ferrer C, Ribas-Fito N, Anto JM, et al. Maternal fish intake during pregnancy and atopy and asthma in infancy. Clin Exp Allergy. 2007;37(4):518-25.

97. Salam MT, Li YF, Langholz B, Gilliland FD. Maternal fish consumption during pregnancy and risk of early childhood asthma. J Asthma. 2005;42(6):513-8.

98. Tabak C, Wijga AH, De Meer G, Janssen NAH, Brunekreef B, Smit HA. Diet and asthma in Dutch school children (ISAAC-2). Thorax. 2006;61(12):1048-53.

99. Antova T, Pattenden S, Nikiforov B, Leonardi GS, Boeva B, Fletcher T, et al. Nutrition and respiratory health in children in six Central and Eastern European countries. Thorax. 2003;58(3):231-6.

100. Arvaniti F, Priftis KN, Papadimitriou A, Papadopoulos M, Roma E, Kapsokefalou M, et al. Adherence to the Mediterranean type of diet is associated with lower prevalence of asthma symptoms, among 10-12years old children: The PANACEA study. Pediatric Allergy and Immunology. 2011;22(3):283-9.

101. Hodge L, Salome CM, Peat JK, Haby MM, Xuan W, Woolcock AJ. Consumption of oily fish and childhood asthma risk. Medical Journal of Australia. 1996;164:137-40.

102. Hoppu U, Rinne M, Lampi AM, Isolauri E. Breast milk fatty acid composition is associated with development of atopic dermatitis in the infant. Journal of pediatric gastroenterology and nutrition. 2005;41(3):335-8.

103. Miyake Y, Sasaki S, Tanaka K, Ohfuji S, Hirota Y. Maternal fat consumption during pregnancy and risk of wheeze and eczema in Japanese infants aged 16-24 months: The Osaka Maternal and Child Health Study. Thorax. 2009;64(9):815-21.

104. Brick T, Schober Y, Böcking C, Pekkanen J, Genuneit J, Loss G, et al. ω-3 fatty acids contribute to the asthma-protective effect of unprocessed cow's milk. Journal of Allergy and Clinical Immunology. 2016;137(6):1699-706e13.

105. Best KP, Gold M, Kennedy D, Martin J, Makrides M. Omega-3 long-chain PUFA intake during pregnancy and allergic disease outcomes in the offspring: A systematic review and meta-analysis of observational studies and randomized controlled trials. American Journal of Clinical Nutrition. 2016;103(1):128-43.

106. Gunaratne AW, Makrides M, Collins CT. Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood. The Cochrane database of systematic reviews. 2015;7:CD010085.

107. Kremmyda LS, Vlachava M, Noakes PS, Diaper ND, Miles EA, Calder PC. Atopy risk in infants and children in relation to early exposure to fish, oily fish, or long-chain omega-3 fatty acids: a systematic review. Clinic Rev Allerg Immunol. 2011;41(1):36-66.

108. Urry Z, Xystrakis E, Richards DF, McDonald J, Sattar Z, Cousins DJ, et al. Ligation of TLR9 induced on human IL-10-secreting Tregs by 1alpha,25-dihydroxyvitamin D3 abrogates regulatory function. J Clin Invest. 2009;119(2):387-98.

109. Netting MJ, Middleton PF, Makrides M. Does maternal diet during pregnancy and lactation affect outcomes in offspring? A systematic review of food-based approaches. Nutrition. 2014;30(11-12):1225-41.

110. Nurmatov U, Devereux G, Sheikh A. Nutrients and foods for the primary prevention of asthma and allergy: Systematic review and meta-analysis. The Journal of allergy and clinical immunology. 2011;127(3):724-33.e30.

111. Lundell AC, Adlerberth I, Lindberg E, Karlsson H, Ekberg S, Åberg N, et al. Increased levels of circulating soluble CD14 but not CD83 in infants are associated with early intestinal colonization with Staphylococcus aureus. Clinical and Experimental Allergy. 2007;37(1):62-71.

112. Johansson I, Hallmans G, Wikman A, Biessy C, Riboli E, Kaaks R. Validation and calibration of food-frequency questionnaire measurements in the Northern Sweden Health and Disease cohort. Public health nutrition. 2002;5(3):487-96.

113. Brekke HK, Jansson PA, Månsson JE, Lenner RA. Lifestyle changes can be achieved through counseling and follow-up in first-degree relatives of patients with type 2 diabetes. J Am Diet Assoc. 2003;103(7):835-43.

114. Administration TSNF. Weight table 2001 [

115. Jonsson K, Barman M, Moberg S, Sjöberg A, Brekke HK, Hesselmar B, et al. Fat intake and breast milk fatty acid composition in farming and nonfarming women and allergy development in the offspring. Pediatric research. 2016;79(1):114-23.

116. Conway JM, Ingwersen LA, Vinyard BT, Moshfegh AJ. Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and nonobese women. Am J Clin Nutr. 2003;77(5):1171-8.

117. Wikland KA, Luo ZC, Niklasson A, Karlberg J. Swedish population-based longitudinal reference values from birth to 18 years of age for height, weight and head circumference. Acta paediatrica (Oslo, Norway : 1992). 2002;91(7):[d]739-54.

118. Nordic Nutrition Recommendations 2012. Integrating nutrition and physical activity. 5th edition ed. Copenhagen: Nordic Council of Ministers; 2012.

119. Francois CA, Connor SL, Wander RC, Connor WE. Acute effects of dietary fatty acids on the fatty acids of human milk. Am J Clin Nutr. 1998;67(2):301-8.

120. Lee CM, Trevino B, Chaiyawat M. A simple and rapid solvent extraction method for determining total lipids in fish tissue. J AOAC Int. 1996;79(2):487-92.

121. Kaluzny MA, Duncan LA, Merritt MV, Epps DE. Rapid separation of lipid classes in high yield and purity using bonded phase columns. J Lipid Res. 1985;26(1):135-40.

122. Lepage G, Roy CC. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. J Lipid Res. 1984;25(12):1391-6.

123. Williams HC. Diagnostic criteria for atopic dermatitis [19]. Lancet. 1996;348(9038):1391-2.

124. eHanifin J, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol. 1980;Suppl. 92:44-7.

125. Strömbeck A, Rabe H, Lundell AC, Andersson K, Johansen S, Adlerberth I, et al. High proportions of FOXP3+CD25high T cells in neonates are positively associated with allergic sensitization later in childhood. Clin Exp Allergy. 2014;44(7):940-52.

126. Bylesjö M, Rantalainen M, Cloarec O, Nicholson JK, Holmes E, Trygg J. OPLS discriminant analysis: Combining the strengths of PLS-DA and SIMCA classification. J Chemometr. 2006;20(8-10):341-51.

127. Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS). J Chemometr. 2002;16(3):119-28.

128. Wells H-G. Studies on the chemistry of anaphylaxis III. Experiments with isolated proteins, especially those of the hens egg. J Infect Dis. 1911;8:147-53.

129. Filipiak B, Zutavern A, Koletzko S, von Berg A, Brockow I, Grübl A, et al. Solid Food Introduction in Relation to Eczema: Results from a Four-Year Prospective Birth Cohort Study. J Pediatr. 2007;151(4):352-8.