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NUTRITIONAL FACTORS AND ALLERGIC DISEASE: FROM INFANCY TO ADOLESCENCE

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Nutritional factors and Allergic disease:

From Infancy to Adolescence

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Truly, madly, deeply.

SUMMARY

Allergic disease is one of the most common non-communicable diseases in childhood. Both overweight and diet have been hypothesized to influence the risk of allergic disease. The aim of this thesis was to investigate the associations between overweight, fish intake and polyunsaturated fatty acids (PUFAs) and allergic disease throughout childhood. All analyses were performed on data from the Swedish prospective birth cohort BAMSE.

The association between body mass index (BMI) status (as a measure of overweight) and risk of asthma at age 8 years was explored among 2,075 children (**study I**). High BMI at ages 1, 1.5, 4 and 7 years were associated with an increased risk of asthma at age 8 years. However, children with high BMI during early childhood but whose BMI normalized before age 7 years had no increased risk of asthma. In contrast, children with a high BMI at age 7 years had an increased risk of asthma, regardless of their earlier BMI. Moreover, a high BMI at age 7 years was associated with an increased risk of aeroallergen sensitization at age 8 years.

Fish intake in relation to subsequent allergic disease was investigated in **studies II** and **III**. A regular fish intake (≥2-3 times/month) at age 1 year reduced the risk of allergic disease up to age 12 years in analyses of 3,285 children (**study II**). Restriction of the analyses to children without early symptoms of allergic disease weakened the inverse associations with asthma and eczema, but the association with rhinitis was unaffected.

Regular fish consumption at school age was assessed in relation to the development of IgE-associated and non-IgE-associated rhinitis between ages 8 and 16 years in analyses of 1,590 children (**study III)**. For total fish intake, cod or fish fingers no significant associations were observed. In contrast, a regular intake of oily fish (≥1 time/week) was associated with a reduced risk of developing rhinitis between ages 8 and 16 years, also after adjustment for infant fish intake and early symptoms of allergic disease.

The association between PUFAs at age 8 years and risk of allergic disease was investigated in **studies III** and **IV**. Calculated intake of total PUFA, α-linolenic acid (18:3 n-3), total n-6 fatty acids, linoleic acid (18:2 n-6), and arachidonic acid (AA, 20:4 n-6) was not associated with rhinitis in **study III**. Meanwhile, higher intake of total very long-chain n-3 fatty acids (VLC n-3 fatty acids, the sum of eicosapentaeonic acid [20:5 n-3], docosapentaeonic acid [22:5 n-3], and docosahexaeonic acid [22:6 n-3]), was associated with a reduced risk of developing non-IgE-associated rhinitis between ages 8 and 16 years.

The composition of PUFAs was measured in plasma phospholipids for a subsample of 940 children. Increasing proportion of total VLC n-3 fatty acids was associated with a reduced risk of asthma, rhinitis and aeroallergen sensitization at age 16 years in **study IV**. In addition, total VLC n-3 fatty acids was associated with a reduced risk of developing asthma between ages 8 and 16 years. AA was associated with a reduced risk of asthma and aeroallergen sensitization at age 16 years. The inverse associations between total VLC n-3 fatty acids and AA and allergic disease were most pronounced for the IgE-associated phenotypes.

In conclusion, the results in this thesis imply that modifiable factors influence the risk of allergic disease in childhood. Early-transient high BMI does not seem to increase the risk, while persistent and late high BMI seem to be associated with concurrent asthma and aeroallergen sensitization at school age. Moreover, fish intake, both in infancy and childhood, and PUFAs, especially VLC n-3 fatty acids, were associated with a reduced risk of subsequent allergic disease throughout childhood.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Förekomsten av allergisk sjukdom har ökat kraftigt under de senaste årtiondena och 30-40% av världens befolkning är drabbad. Astma, hösnuva och eksem är exempel på allergisk sjukdom, många av barnen i Sverige kommer att ha minst en av dessa åkommor under barndomen. Två faktorer som ökar risken för allergisk sjukdom hos barn är ärftlighet och att utsättas för rökning under fosterstadiet eller som nyfödd. Ytterligare en mängd miljö- och livsstilsfaktorer, inklusive luftföroreningar, övervikt och kost, kan möjligtvis spela roll för risken att insjukna och intensiv forskning pågår. Förekomsten av övervikt ökade under samma tidsperiod som förekomsten av allergisk sjukdom. Det verkar finnas ett samband mellan övervikt och framförallt astma, men det är inte klarlagt om övervikt leder till ökad risk för astma eller tvärtom.

Fisk är ett livsmedel som innehåller många olika näringsämnen. Framför allt fet fisk har ett högt innehåll av både vitamin D och långkedjiga omega-3 fettsyror. Dessa har visat sig ha egenskaper som kan påverka immunförsvaret och skulle därigenom kunna minska risken för allergisk sjukdom. Effekterna av omega-3 fettsyror påverkas av nivåerna av omega-6 fettsyror som verkar ha både positiva och negativa effekter på immunförsvaret, vilket innebär att det är viktigt att studera dessa tillsammans.

Syftet med denna avhandling var att undersöka sambanden mellan övervikt, fiskkonsumtion, samt omega-3 och omega-6 fettsyror och risken för allergisk sjukdom under barndomen. Detta gjordes med hjälp av data från födelsekohorten BAMSE, som står för ”Barn, Allergi, Miljö, Stockholm, en Epidemiologisk studie”, och inkluderar 4089 barn som har följts från 2 månaders ålder upp till 16 års ålder. Kohorten är fortfarande aktiv och nästa uppföljning är planerad vid 22 års ålder.

I den **första delstudien** använde vi body mass index (BMI, kg/m^2) som ett mått på övervikt. Vi undersökte hur ett högt BMI vid åldrarna 1 år, 1,5 år, 4 år och 7 år, samt hur förändringen av BMI över tid påverkade risken för astma och allergisk sensibilisering vid 8 års ålder hos 2075 barn. Vi såg att ett högt BMI vid åldrarna 1 år, 4 år och 7 år var kopplat till en högre risk för att ha astma vid 8 års ålder. När vi undersökte förändringen av BMI över tid kunde vi däremot se att barn som endast hade ett högt BMI vid de tidigare åldrarna och sedan utvecklade ett normalt BMI till 7 års ålder inte hade någon ökad risk, medan barn som hade ett högt BMI vid 7 års ålder hade en ökad risk för att ha astma och allergisk sensibilisering oavsett tidigare BMI status.

I den **andra delstudien** undersökte vi sambandet mellan intag av fisk vid 1 års ålder och risken för allergisk sjukdom från 1 år upp till 12 års ålder hos 3285 barn. Vi såg en minskad risk för astma, hösnuva och eksem hos de barn som åt fisk minst 2-3 gånger i månaden vid 1 års ålder. Allergiska symtom tidigt i livet kan påverka föräldrarnas benägenhet att servera allergena livsmedel till barnet. Eftersom vi var oroliga att sådan sjukdomsrelaterad påverkan

kunde ha skett för fiskintaget vid 1 års ålder valde vi att i ett nästa steg utesluta de barn som haft eksem eller väsande andning innan 1 års ålder. Sambanden för astma och eksem påverkades av uteslutningen medan den minskade risken för hösnuva var oförändrad. Sambandet mellan ett regelbundet fiskintag vid 1 års ålder och hösnuva upp till 12 års ålder påverkades inte heller av att vi i den statistiska modellen tog hänsyn till fiskintaget vid 8 års ålder.

I den **tredje delstudien** utgick vi från barn utan hösnuva vid 8 års ålder och analyserade sambanden mellan uppgett fiskintag samt beräknat omega-3 och omega-6 intag vid 8 års ålder och risken att insjukna i hösnuva mellan 8 år och 16 års ålder bland 1590 barn. Inga samband hittades mellan totalt fiskintag, mager fisk eller fiskpinnar och nyinsjuknande i hösnuva. Däremot hade de barn som åt fet fisk minst en gång i veckan vid 8 års ålder en lägre risk för att insjukna i hösnuva mellan 8 år och 16 års ålder. Likaså hade de barn med högst intag av långkedjiga omega-3 fettsyror en lägre risk för att insjukna i hösnuva. För omega-6 fettsyror sågs inga samband.

För den **fjärde delstudien** mättes nivåerna av fettsyror i blodprover från 940 barn vid 8 års ålder. Sambanden mellan blodnivåer av omega-3 och omega-6 fettsyror och risken för att ha eller drabbas av astma, hösnuva eller luftburen sensibilisering vid 16 års ålder undersöktes. Högre nivåer av långkedjiga omega-3 fettsyror minskade risken för att ha astma, hösnuva och luftburen sensibilisering vid 16 års ålder samt risken för att insjukna i astma mellan 8 år och 16 års ålder. Även högre nivåer av omega-6 fettsyran arakidonsyra minskade risken för att ha astma och luftburen sensibilisering vid 16 års ålder.

Sammanfattningsvis visar resultaten i denna avhandling att förändringsbara livsstilsfaktorer kan påverka risken för att ha eller insjukna i allergisk sjukdom under barndomen. Resultaten för BMI visar att det är viktigt att motverka fortsatt övervikt efter småbarnsåren. Att fiskkonsumtion både tidigt i livet och senare under barndomen verkar minska risken för allergisk sjukdom visar vikten av en god kosthållning och stödjer de befintliga kostråden om ett regelbundet fiskintag som inkluderar både mager och fet fisk.

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I. **Öhman Magnusson J**, Kull I, Mai XM, Wickman M, Bergström A. Early childhood overweight and asthma and allergic sensitization at 8 years of age. *Pediatrics* 2012;129:70-76.

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IV. **Magnusson J**, Ekström S, Kull I, Håkansson N, Nilsson S, Wickman M, Melén E, Risérus U, Bergström A. Polyunsaturated fatty acids in plasma at 8 years and subsequent allergic disease. (*manuscript*)

These publications are referred to as **studies I-IV**, and reproduced in full at the end of the thesis.

RELATED PUBLICATIONS

(Not included in the thesis)

I. Rosenlund H, **Magnusson J**, Kull I, Håkansson N, Wolk A, Pershagen G, Wickman M, Bergström A. Antioxidant intake and allergic disease in children. *Clin Exp Allergy* 2012;42:1491-1500.

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VI. Besharat Pour M, Bergström A, Bottai M, **Magnusson J**, Kull I, Moradi T. Age at adiposity rebound and body mass index trajectory from early childhood to adolescence; differences by breastfeeding and maternal migration background. *Pediatr Obes* 2016 (Epub ahead of print)

VII. Ekström S, **Magnusson J**, Kull I, Andersson N, Bottai M, Besharat Pour M, Melén E, Bergström A. Body mass index development and asthma throughout childhood. *Accepted manuscript*

CONTENTS

LIST OF ABBREVIATIONS

AA	Arachidonic acid
ALA	α-Linolenic acid
APC	Antigen-presenting cell
AR	Allergic rhinitis (IgE-associated)
ASM	Airway smooth muscle
BMI	Body mass index
CI	Confidence interval
DHA	Docosahexaeonic acid
DPA	Docosapentaeonic acid
EPA	Eicosapentaeonic acid
FFQ	Food frequency questionnaire
GEE	Generalized estimating equations
GERD	Gastroesophageal reflux disease
IgE	Immunoglobulin E
IOTF	International obesity task force
IQR	Interquartile range
kU/L	Kilounits per liter
LA	Linoleic acid
NAR	Non-allergic rhinitis (non-IgE-associated)
OR_{adj}	Adjusted Odds ratio
PGs	Prostaglandins
PUFAs	Polyunsaturated fatty acids
VLC n-3	Very long-chain n-3
WHO	World Health Organization

1 INTRODUCTION

Allergic disease affects 30-40% of the population worldwide.[1] The prevalence of allergic disease in childhood has increased rapidly during the last decades,[2-5] and has become one of the most common non-communicable diseases in childhood affecting 25% of the children in Europe.[1]

Genetic predisposition explains part of the risk for allergic disease. Nevertheless, the rapid increase in prevalence implies that other factors also play a role; the environment and lifestyle must influence the risk of allergic disease as well. Overweight is one modifiable factor that has been proposed to affect the risk of asthma in particular. There has been a dramatic increase in the prevalence of overweight during the last decades, coincidental with the increase in allergic disease. Uncertainties remain as to whether overweight causes asthma or if it is the other way around.

Diet is another modifiable factor hypothesized to affect the risk of allergic disease. There has been a shift in dietary intake from high consumption of butter and fish to high consumption of margarines and vegetable oils.[6] This shift has led to lower consumption of saturated and very long-chain n-3 polyunsaturated fatty acids and to higher consumption of n-6 polyunsaturated fatty acids. Very long-chain n-3 and n-6 polyunsaturated fatty acids are found to have immuno-modulatory effects and might affect the risk of allergic disease in opposite directions, with a protective effect of n-3 polyunsaturated fatty acids and an adverse impact of n-6 polyunsaturated fatty acids.[7]

The aim of this thesis was to study the influence of overweight, fish intake and n-3 and n-6 polyunsaturated fatty acids on the risk of allergic disease throughout childhood.

2 BACKGROUND

2.1 CHILDHOOD ALLERGIC DISEASE

Allergic disease is characterized by an overreaction of the immune system to a foreign substance, harmless for most people. Allergic diseases, such as asthma, rhinitis and eczema may be triggered by allergens. The process has two steps. In the first step, the person encounters an allergen which is taken up by antigen-presenting cells (**Figure 2.1**). This will, through multiple actions, stimulate B-cells to produce allergen-specific immunoglobulin E (IgE) antibodies. The allergen-specific IgE antibodies will then be released and bind to IgE receptors on mast cells and basophils, the person becomes sensitized.[8] In the next step, when the person is exposed to the same allergen again, the specific IgE antibodies recognize the allergen, which leads to the release of inflammatory mediators and an allergic reaction. Common allergens are proteins in food and pollens, house dust mite and dander from animals. In addition to IgE-mediated allergic disease, children can also suffer from non-IgE-mediated allergic disease. The triggers of this phenotype are less known.

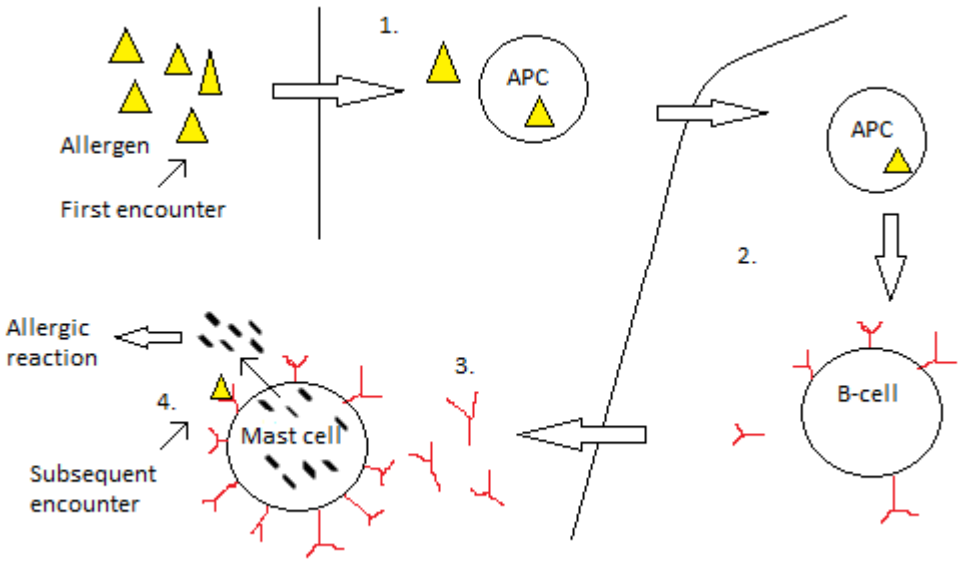

Figure 2.1 A simplified overview of the sensitization process and allergic reaction. A person encounters an allergen, which passes the epithelial barrier and is taken up by an antigen-presenting cell (APC) (1). The APC with the allergen will, through multiple actions, stimulate B-cells in the lymph node to produce allergen-specific immunoglobulin E (IgE) antibodies (2). The allergen-specific IgE antibodies are released and bind to IgE-receptors on mast cells; the person becomes sensitized (3). The next time a person encounters the same allergen it will be recognized by the allergen-specific IgE on the mast cell; this will lead to release of inflammatory mediators, such as histamine, and an allergic reaction (4).

A child with allergic disease often suffers from more than one of these entities during childhood and multi-morbidity is common.[9] A concept often mentioned is the "allergic march". This concept refers to the natural sequence of allergic disease, with eczema as the starting point in infancy and early childhood, and later on asthma and rhinitis are developed.[10]

2.1.1 Definition and diagnosis

2.1.1.1 Asthma

Asthma is a disease of the airways. It is often triggered by IgE-sensitization, but frequently evolves to chronic airway inflammation unrelated to allergen contact.[11] Symptoms of asthma are dyspnea, wheezing, raspy breathing and coughing. Clinically, asthma is often diagnosed through the clinical history. In epidemiological studies, questionnaires are often used. A doctor's-diagnosis is often asked for and in combination with questions on symptoms and use of medication it is possible to define previous and current disease. In toddlers, it may be troublesome to define asthma, since they often suffer from upper respiratory tract infections with similar symptoms.

2.1.1.2 Rhinitis

Rhinitis is characterized by inflammation in the nose leading to nasal blockage, sneezing and a runny, itchy nose.[12] In the clinic, rhinitis is diagnosed by symptom history combined with an examination of the nose. In epidemiological studies, symptom questionnaires are used. Questions can cover symptoms following contact with allergens such as furred pets and pollens, and also in which season of the year the symptoms have occurred and for how long.

2.1.1.3 Eczema

Eczema is an inflammatory disease of the skin; the skin barrier is disrupted and this leads to dry skin and itchy rashes.[13] Eczema can be diagnosed by ocular inspection. In epidemiological studies, questionnaires regarding symptoms, ointment usage and possible doctor's diagnosis are usually used to define eczema.

2.1.1.4 Allergic sensitization

Occurrence of IgE antibodies in the blood shows if a person is sensitized. A person can be sensitized to different allergens, e.g., from foods, furred animals or pollens. Sensitization is clinically tested by skin prick test or by measuring the level of IgE antibodies in the blood. Single allergens or a mix of allergens can be tested. Phadiatop® is such a mix, which contains antibodies of furred pets, pollens, mold and mite, while the mix fx5® contains antibodies to the 6 most common food allergen extracts. Being sensitized does not mean that a person may experience allergic symptoms at exposure, but the higher the IgE level, the higher the likelihood of symptoms.

2.1.2 Occurrence

The prevalence of allergic disease in childhood has risen during the last decades.[3, 4] In the large international multi-center study ISAAC phase III an increase in the prevalence of allergic disease over a 10 year period from the early 1990s to the beginning of the 21st century was observed.[3] A British study, which assessed the prevalence of allergic disease among children repeatedly during 40 years until 2004, observed a steady increase from the

middle of the 1960s until the end of the 1990s.[4] After the 1990s the increase in prevalence of asthma seems to have leveled off, while a continued rise was observed for rhinitis and eczema. In the first phase of the ISAAC study large differences in prevalence of allergic disease were observed between regions.[14] Although regional differences exist, 30-40% of the global population is affected by allergic disease, according to the World Allergy Organization,[1] and asthma is the most common chronic disease among children according to the World Health Organization (WHO).[15]

In Sweden, the pattern of change has been similar to the changes observed worldwide. The prevalence of childhood allergic disease in Sweden doubled between the late 70s and the early 90s.[2] Since then the prevalence seems to be stable,[3, 5] or there might be a continued increase in the prevalence of rhinitis among both school-aged children and adolescents.[16, 17] The prevalence of asthma in school-aged children was reported to be between 6-10% in the 21st century, while the prevalence rates of rhinitis and eczema seem to be around 7-15%, and 19-26%, respectively.[3, 5, 16] Even higher prevalence of rhinitis, in particular, has been reported in adolescents (26% vs. 7-15% at earlier ages).[17]

2.1.3 Consequences

The consequences of childhood allergic disease occur both on individual and societal level. First, on the individual level allergic disease can lead to sleep disturbance, impaired quality of life and absence from school.[1, 11, 18, 19] For children with rhinitis, 2 million school-days are lost annually in the United States alone, according to the World Allergy Organization.[1]

Second, on the societal level, allergic disease has direct and indirect effects on the economy.[20] Direct costs are due to the need for visits to a physician, laboratory tests and medication, while indirect costs depend on absenteeism from school and, for parents, from workplaces.

It is important to try to prevent and manage childhood allergic disease because, in addition to the burden on the child, many children seem to retain with their disease into adulthood and suffer from it throughout life.[21]

2.1.4 Risk and protective factors

Some of the proposed risk and protective factors are covered briefly in this section. The topics of childhood overweight, fish, and polyunsaturated fatty acids (PUFAs) are described separately in later sections.

Parental allergic disease has been established to increase the risk of allergic disease in children.[22, 23] In addition, specific genes have been found to influence the risk of asthma, rhinitis and eczema, respectively.[24] Sex has been suggested to influence the risk of allergic disease. Asthma is more common among boys than girls at younger ages, while there is a

shift to a higher prevalence among girls than boys during adolescence.[25] Rhinitis increases with age in both sexes until adolescence, then the increase continue in girls but not in boys.[26] The changes in occurrence of asthma and rhinitis during adolescence have been proposed to depend on puberty and hormonal changes. In contrast, eczema is most common among girls throughout the childhood years.[27] Second hand tobacco smoke exposure, often assessed as maternal smoking during pregnancy or parental smoking in infancy, has consistently been associated with an increased risk of allergic disease during childhood.[22, 28-30] In addition, preterm birth has been associated with an increased risk of asthma.[22, 30]

Maternal characteristics during pregnancy, such as overweight or obesity, and diet, have been hypothesized to influence the offspring's risk of developing allergic disease. Maternal overweight or obesity might increase the offspring's risk of asthma especially.[22, 31] However, some of the association might be explained by the child's own overweight.[31] Childhood overweight in relation to allergic disease is described further in sections 2.2.3 and 2.2.4. In a recent meta-analysis of maternal dietary intake during pregnancy, inverse associations between vitamin E and zinc, and also vitamin D, and wheeze in offspring were identified; however, there were no consistent findings for maternal consumption of fruit, vegetables and other foods.[32] In another recent meta-analysis of observational studies and randomized controlled trials, the conclusion was that maternal fish consumption and supplementation with very long-chain n-3 (VLC n-3) fatty acids during pregnancy have a suggestive influence on a reduced risk of allergic disease within the first 12 months of the child's life.[33] In addition, in a long-term follow-up of a randomized controlled trial a reduced risk of asthma in the group of adult offspring to mothers supplemented with VLC n-3 fatty acids compared with offspring to mothers supplemented with olive oil was observed.[34] In contrast, in a Cochrane review the conclusion was that there was limited evidence to introduce maternal supplementation during pregnancy to reduce the risk of allergic disease in offspring.[35] No consistent evidence for an association between maternal consumption of fish and n-3 fatty acids during pregnancy and reduced risk of allergic disease in offspring was reported in another recent meta-analysis of maternal diet during pregnancy.[32]

Breastfeeding has also been hypothesized to influence the risk of allergic disease in offspring, however, results from epidemiological studies on breastfeeding duration have been conflicting.[36] The timing of introduction of solid allergenic foods to the infant's diet, for example, egg, peanut, fish, and cow's milk, has been thought to play a role for the risk of allergic disease for a long time. The earlier belief was to delay the introduction of allergenic foods to reduce the risk of allergic disease.[37] However, this strategy was unsuccessful and the focus shifted to oral tolerance and early introduction.[38] Results from recent interventions on early introduction of peanuts have prompted an increased interest in this research field.[39]

Already in 1989, Strachan suggested that better hygiene with fewer infections in early childhood was a possible explanation for the observed increase in allergic disease.[40] However, infections in early childhood have been observed to increase the risk.[41] The

hypothesis has later been reformulated; a decreased exposure to a diversity of environmental microbes might lie behind the increased prevalence of allergic disease.[42, 43] Factors that have been studied, related to this hypothesis, are number of siblings, living on a farm, day care attendance, pet ownership, and socioeconomic status. The gut microbiome might also play a role for the risk of childhood allergic disease; lower microbiota diversity in infancy has been associated with development of allergic disease.[44] Furthermore, supplementation with probiotics seems to reduce the risk of eczema, but the evidence is more inconclusive for other allergic diseases.[44]

Traffic-related air pollution might increase the risk of childhood asthma.[22, 45, 46] In two recent meta-analyses the conclusion was that early exposure to traffic-related air pollution seems to increase the risk of developing asthma during childhood; the magnitude of the association increased with increasing age.[45, 46] Exposure to mold or fungi in the indoor environment might increase the risk of childhood asthma.[22] Findings for other allergic diseases are more inconsistent.

These are some of the factors proposed to influence the risk of allergic disease in childhood. Most of them are related to the environment or lifestyle and potentially modifiable, suggesting that there are ways to decrease the risk. Some of the factors are more established than others, such as parental allergic disease, genetic predisposition, exposure to second hand tobacco smoke and prematurity.[22]

2.2 CHILDHOOD OVERWEIGHT

2.2.1 Definition and measurements

Overweight is defined as excess of body fat to an extent that affects health.[47, 48] An imbalance in energy intake and expenditure that leads to a positive energy balance results in accumulation of body fat.

A widely used tool to define overweight in epidemiological studies is body mass index (BMI). BMI is calculated as weight in kilograms divided by height in meters squared (kg/m^2). BMI is easy to use and has been shown to identify overweight in children to a high extent.[49, 50] To identify overweight in children with BMI different reference values can be used.[51] The International Obesity Task Force (IOTF) constructed reference values for children corresponding to the adult cut offs for overweight and obesity.[52, 53] WHO has constructed international BMI-for-age growth charts for children.[54, 55] In addition, many countries have their own national BMI-for-age growth charts based on the child population, often using the 85^{th} percentile as cut off for overweight and the 95^{th} percentile as cut off for obesity.[51] In epidemiological studies, another method is to use the 85^{th} and 95^{th} percentile based on the children in a cohort. The observed prevalence can differ depending on the reference choice, therefore it is important to state chosen reference methods and cut offs used.

2.2.2 Occurrence

In 2014, there were around 41 million preschool aged children who were overweight or obese worldwide, according to the WHO.[56] The prevalence of overweight has risen dramatically in the most recent decades.

In a study based on children below 10 years of age from eight European countries, taking place 2007-2010, an overweight prevalence (defined by the IOTF reference values) above 40% in southern Europe compared with less than 10% in northern Europe, including Sweden, was observed.[57] Among 10-11 year old children in Sweden, the observed prevalence of overweight (defined by the IOTF reference values) was higher (17-24% vs. 10% in younger children), but might have reached a plateau.[58] However, the prevalence can differ widely depending on socio-economic status, as seen both in Europe and in Sweden; population groups with lower socio-economy have higher prevalence rates of overweight and obesity in general.[57, 58]

2.2.3 Mechanisms linking overweight and allergic disease

The concurrent epidemic of asthma and overweight led to theories about an association between them. Observed associations between overweight and asthma might be due to over-diagnosis of asthma among overweight subjects. However, a number of possible mechanisms to explain how overweight might cause asthma have been put forward (**Table 2.1**).[59, 60] Some of the suggested mechanisms can also explain a possible link between overweight and allergic sensitization. It has also been proposed that overweight and asthma might be determined by common genetics and lifestyle factors.[61] Examples of shared lifestyle factors that have been suggested are prenatal exposures, e.g., maternal intake of antioxidants and n-3 and n-6 fatty acids, the child's own intake of n-3 and n-6 fatty acids, the microbiota, and sedentary behaviors.

Table 2.1 Hypothesized mechanisms suggested to explain how overweight might cause asthma[59, 60]

Mechanisms suggested:	Examples:
Systemic inflammation caused by fat mass-derived molecules	Leptin and cytokines (TNF-alpha & IL-6 etc.) released from fat mass into the blood leads to inflammatory activation at other sites, for example the lungs. Adiponectin, which has anti-inflammatory effects, declines in obesity
Systemic inflammation caused by obesity-related oxidative stress	Oxidative stress might cause airway oxidative stress and inflammation leading to asthma
Obesity co-morbidities	GERD[1], Type-2 diabetes trough insulin resistance
Obesity effects on lung mechanics	Low tidal volume caused by obesity leads to small ASM[2] strain, which gives greater stiffness in ASM, leading to even less ASM strain - a bad downward spiral resulting in substantial ASM contraction and airway narrowing

1. GERD=Gastroesophageal reflux disease
2. ASM=Airway smooth muscle

2.2.4 Observational studies of overweight and allergic disease

One cross-sectional study, using self-reported weight and height, did not observe an association between BMI, used as a proxy for overweight, and asthma.[62] A few cross-sectional studies did not observe an association with asthma but with wheeze,[63] or exercise-induced respiratory symptoms.[64] Most cross-sectional studies have observed an association between a high BMI, and asthma in childhood.[65-71] However, it is impossible to draw any conclusions about temporal relationships from cross-sectional studies. The longitudinal studies performed observed a high BMI to be associated with an increased risk of asthma.[72-78] Some of these investigated increase in BMI over time and observed an increased risk of asthma.[73, 74, 77] None of these studies have been able to assess BMI repeatedly from early childhood until end of follow-up and changes besides increase in BMI have not been investigated. Only one study examined change in BMI status and the risk of asthma in children and observed no association between an early-transient high BMI and asthma later in childhood, on the other hand, current high BMI was associated with an increased risk of coincident asthma.[79] These results indicated that the pattern of change in overweight status is of importance to disentangle the association between overweight and asthma. A potential association between a high BMI and allergic sensitization was investigated in cross-sectional analyses with inconclusive results.[63, 64, 66, 69, 80, 81] In two longitudinal studies, no association between a high BMI and subsequent allergic sensitization was observed.[78, 79] The association between overweight and asthma and allergic sensitization needed further investigation in longitudinal studies with repeated measurement of overweight starting in early childhood.

2.3 DIETARY INTAKE

"Let food be thy medicine and medicine be thy food"-Hippocrates

Everyone has to eat. What you eat and how you eat affects many parts of your life. All the way back to ancient Greece, people have been interested in diet and have thought that it affects your health. The ancient Greeks believed that you could avoid becoming sick or get better by eating healthy. Today, we have national dietary recommendations that are supposed to reduce the risk of disease in the population, and there are different hypotheses relating diet to allergic disease. Two parallel hypotheses, with a focus on antioxidants and fat quality, were suggested during the 1990s.[6, 82] Both hypotheses rose from an observed change in consumption during the preceding decades, coincident with the rise in allergic disease. In addition, both hypotheses have been studied during different developmental phases; in pregnancy, infancy and childhood. Seaton et al. suggested that a decline in fruit and vegetable intake lowered people's antioxidant status, leading to a population more susceptible to the oxidative load from various exposures, resulting in airway inflammation.[82] The evidence from observational studies with a focus on children's own intake of antioxidants or antioxidant-rich foods has been inconclusive.[22, 83]

The hypothesis regarding fat quality was suggested by Black and Sharp.[6] A decreased consumption of butter and fish and an increased consumption of margarines have led to reduced amounts of saturated fat and n-3 PUFAs and an increased amount of n-6 PUFAs in the diet; these changes were suggested to be the reasons for the rise in asthma and allergy.[6]

2.3.1 Fish intake

2.3.1.1 Background

In the Nordic Nutrition Recommendations, an increased consumption of fish is encouraged to promote energy balance and health.[84] The Swedish National Food Agency recommends consumption of fish at least 2 times per week with inclusion of both lean and oily fish.[85] Lean types are for example cod, pollock, and pike, while salmon, herring, and mackerel are examples of oily fish. The advices regarding introduction of fish into the infant's diet have changed over the years. Previously, the advice was to delay the introduction of fish for infants at increased risk of allergic disease, however, the avoidance was not effective and the recommendation has been removed. Fish is a good source of several nutrients, and especially oily fish contain high levels of VLC n-3 fatty acids, which are hard to obtain in sufficient amounts from other dietary items. However, it is important to recognize that oily fish, in particular, can contain high amounts of dioxins and polychlorinated biphenyls, depending on where the fish has been caught. Therefore, children and fertile women are advised to avoid wild-caught oily fish from certain areas. Another aspect in choice of fish is to choose species not threatened by over-fishing and which are caught or farmed in an environmentally-friendly way. These aspects will not be discussed further in this thesis.

2.3.1.2 Nutritional content

Nutrients in fish that might influence the risk of allergic disease are selenium, vitamin D and VLC n-3 fatty acids.

Selenium seems to have both antioxidative and immuno-modulatory properties and has been suggested to decrease the risk of allergic disease in childhood.[86] However, the evidence from epidemiological and intervention studies has not been convincing.[87-89]

Vitamin D has been shown to affect the immune system in several ways.[90] Studies on the association between vitamin D status and asthma have been inconclusive.[91-94] Few studies have investigated a potential association between vitamin D and rhinitis; one study found an inverse association between vitamin D status in childhood and subsequent rhinitis.[92]

VLC n-3 fatty acids eicosapentaeonic acid (EPA, 20:5 n-3), docosapentaeonic acid (DPA, 22:5 n-3) and docosahexaeonic acid (DHA, 22:6 n-3) are present in high amounts in oily fish and have been hypothesized to decrease the risk of allergic disease (see more below).

2.3.1.3 Observational studies of fish intake in childhood and allergic disease

Introduction and consumption of fish in infancy have consistently been associated with a reduced risk of allergic disease in preschool ages.[95-102] However, there have been no longitudinal studies which had investigated a potential association beyond preschool age.

Childhood fish intake was associated with allergic disease in a number of cross-sectional studies,[103-112] with some exceptions.[113-117] None of these studies found inverse associations specifically for rhinitis.[104, 107, 113, 115, 116] One longitudinal study has been performed, focusing on the risk of asthma and allergic sensitization, and found no association between fish intake during childhood and the outcomes up to age 8 years.[118] Few of the previous studies have distinguished between types of fish, which is of importance if we believe that the biological mechanism is mediated through vitamin D or VLC n-3 fatty acids abundant in oily fish. There was clearly a need for longitudinal studies distinguishing between types of fish.

2.3.2 Polyunsaturated fatty acids

2.3.2.1 n-3 and n-6 families

The n-3 and n-6 families of PUFAs are characterized by the location of their first double bond in the methyl chain. The n-3 family has the first double bond three carbon atoms from the methyl end of the carbon chain and the n-6 family has the first double bond six carbon atoms from the methyl end. N-3 and n-6 fatty acids are involved in many necessary processes in the body.[119] N-3 and n-6 fatty acids are often present in the same foods, but n-6 fatty acids are often present in a higher amount. It has been hypothesized that an elevated intake ratio of n-6 to n-3 fatty acids might increase the risk of cardiovascular and inflammatory diseases.[120]

The n-3 fatty acid α-linolenic acid (ALA, 18:3 n-3) and the n-6 fatty acid linoleic acid (LA, 18:2 n-6) are essential to humans as we cannot synthesize them. The main dietary sources of ALA are vegetable oils, like canola oil, margarines of canola oil, and walnuts. LA is found in vegetable oils, like sunflower, canola, and corn oils, and margarines of these oils. ALA and LA are medium-chain fatty acids and can be elongated to longer fatty acids in the body. Arachidonic acid (AA, 20:4 n-6) is the most common very long-chain n-6 fatty acid and is synthesized from LA in a competing pathway to the VLC n-3 fatty acids (**Figure 2.2**). The most common VLC n-3 fatty acids are EPA and DHA; these are elongated from ALA to some extent (**Figure 2.2**). The conversion both of VLC n-3 fatty acids from ALA[121] and of AA from LA might be low in humans.[122] The main dietary source of VLC n-3 fatty acids is oily fish, while AA is found in eggs and most types of meat.

Blood composition of the essential fatty acids ALA and LA, as well as of EPA and DHA, are considered to be good biomarkers of dietary intake.

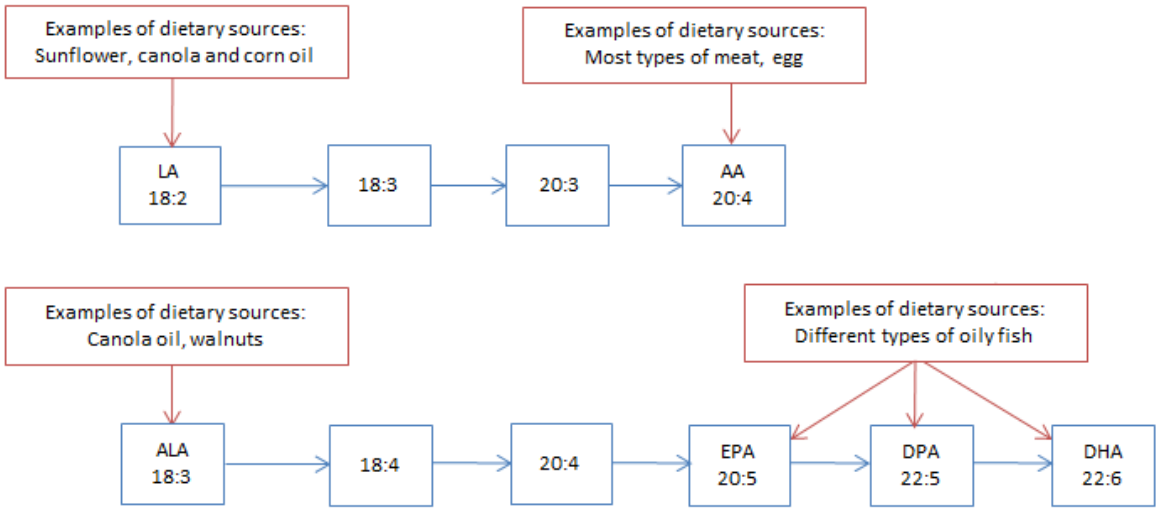

Figure 2.2 Schematic picture of the endogenous pathways of arachidonic acid (AA, 20:4 n-6) from linoleic acid (LA, 18:2 n-6), and of eicosapentaeonic acid (EPA, 20:5 n-3) docosapentaeonic acid (DPA, 22:5 n-3) and docosahexaeonic acid (DHA, 22:6 n-3) from α-linolenic acid (ALA, 18:3, n-3). The two pathways compete for the same desaturases.

2.3.2.2 The influence of very long-chain polyunsaturated fatty acids on allergic disease

VLC n-3 fatty acids have been shown to have anti-inflammatory and inflammatory reducing effects, while AA is the substrate of pro-inflammatory mediators (**Figure 2.3**).[7, 123] They appear to have an influence through incorporation into membrane phospholipids of cells involved in inflammation, like lymphocytes, macrophages and neutrophils.[124] AA was thought of as a pro-inflammatory agent, but has been shown to have anti-inflammatory effects as well (**Figure 2.3**). Both VLC n-3 fatty acids and AA have pleiotropic effects which might influence the risk of allergic disease and which have not all been fully elucidated. Moreover, the effects of n-3 and n-6 fatty acids might be dependent on gene-nutrient

interactions. Genetic variations affect how the PUFAs are metabolized endogenously; this can potentially modulate the influence of these fatty acids on the risk of allergic disease.[125] A few intervention studies with supplementation of VLC n-3 fatty acids to infants have been conducted. The conclusion from these was that despite increases in blood proportions of VLC n-3 fatty acids and possible immunological changes in the blood, no clear evidence of a clinical impact could be proven.[126]

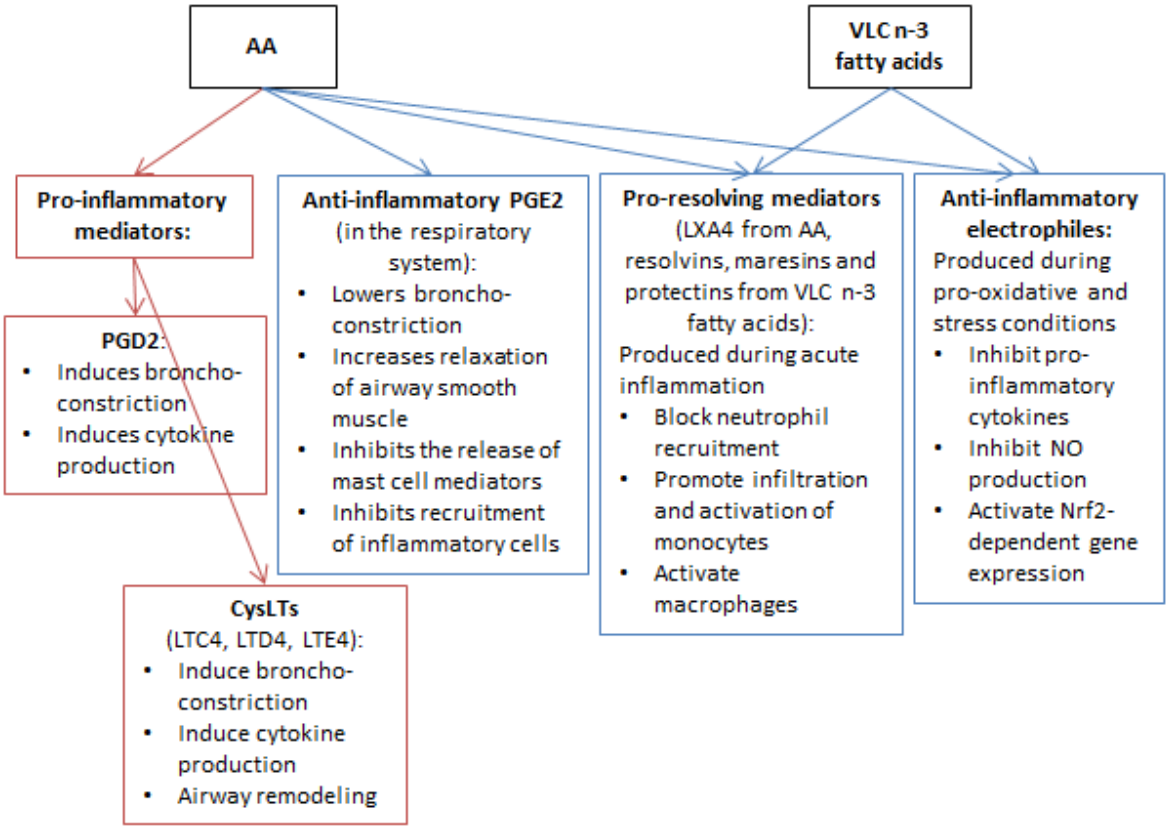

Figure 2.3 Arachidonic acid (AA, 20:4 n-6) and VLC n-3 fatty acids are the substrates for many different inflammatory (red boxes), and anti-inflammatory (blue boxes) mediators. Most effects are not yet fully elucidated.

2.3.2.3 Observational studies of polyunsaturated fatty acids in childhood and allergic disease

There have mainly been cross-sectional studies of the association between PUFA intake and PUFA proportions measured in blood, used as biomarkers of dietary intake and metabolism, and allergic disease in childhood. Studies of calculated dietary intake of n-3 and n-6 PUFAs have not observed a consistent association with allergic disease.[117, 127-129] In contrast, some studies, which used blood proportions of PUFAs, observed a lower level of VLC n-3 fatty acids in subjects with allergic disease than in subjects without allergic disease.[104, 130, 131] However, there have been reports of no differences in blood proportions as well.[112] No prospective studies of the influence of total PUFA and specific PUFAs on subsequent allergic disease up to adolescence had been performed in a population-based cohort.

3 AIM

The overall aim of the project was to evaluate the associations between overweight, fish intake, n-3 and n-6 polyunsaturated fatty acids and allergic disease throughout childhood.

More specifically, we aimed to:

- Examine the associations between body mass index, as a measure of overweight, during the first 7 years of life, and asthma and allergic sensitization at 8 years of age. (**Study I**)

- Investigate the impact of fish consumption in infancy and childhood on prevalent and incident allergic disease up to adolescence. (**Studies II** and **III**)

- Investigate the role of n-3 and n-6 polyunsaturated fatty acids, as calculated intake and as biomarkers in plasma, in relation to prevalent and incident allergic disease up to adolescence. (**Studies III** and **IV**)

4 RESEARCH APPROACH

4.1 SUBJECTS

All studies in this thesis are performed with data from the population-based birth cohort "Barn, Allergi, Miljö, Stockholm, en Epidemiologisk studie" (BAMSE, in English: "Children, Allergy, Milieu, Stockholm, an Epidemiological study"). The primary aim of BAMSE was to study risk factors for allergic disease, such as environmental factors, parental allergic disease, and diet. Newborn babies were recruited through child health centers in four regions in Stockholm County between February 1994 and November 1996 (**Figure 4.1**). The four regions were supposed to reflect both inner city and suburban areas, and catch a socioeconomic gradient.

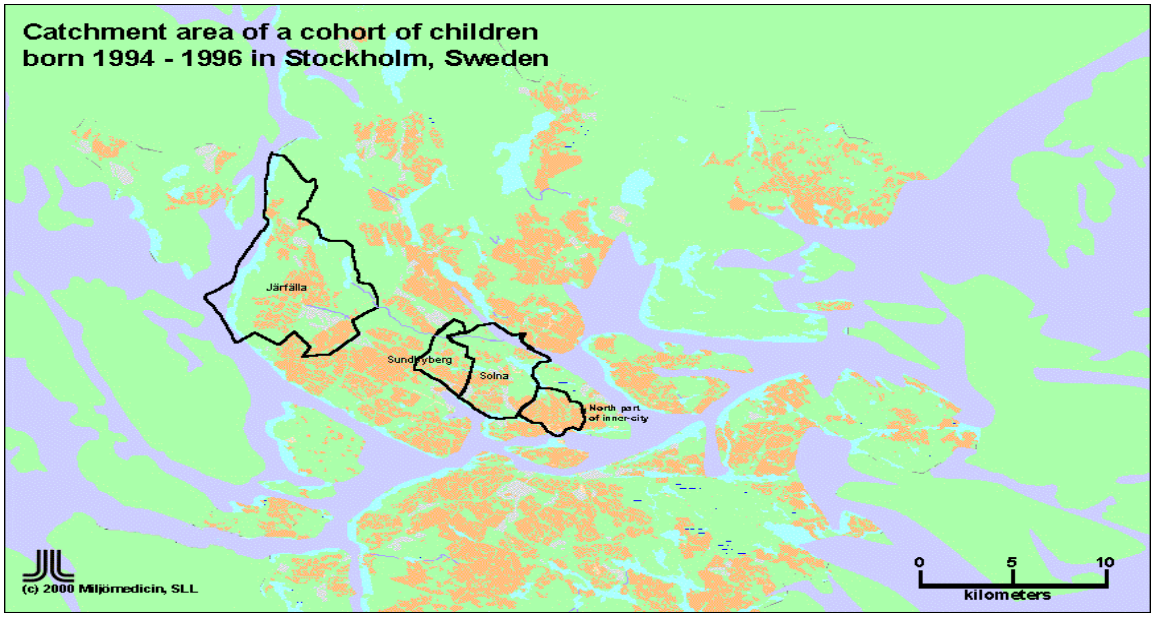

Figure 4.1 A map of the catchment area for the BAMSE cohort. Newborn babies were recruited from four regions in Stockholm County, the regions were supposed to reflect inner city and suburban areas, and to catch a socioeconomic gradient.

During the time period in question, 7,221 children were born in the catchment area, of whom 5,488 were eligible for inclusion in the cohort. The rest could never be reached or were excluded. Reasons for exclusion were that the family planned to move within a year, that the family had a seriously ill child or insufficient knowledge of the Swedish language, or that an older sibling was already enrolled. The parents of 4,089 newborns answered the first mailed questionnaire, when their children were a mean age of 2 months. The cohort of 4,089 children has been followed with repeated questionnaires and clinical examinations over the years (**Figure 4.2**). The next-follow up is under planning and will take place when the participants are 22 years of age. BAMSE is a closed prospective cohort, meaning that no new children have been included over time. The families have had the possibility to skip a follow-up and still be contacted for the next, as long as they did not decline further participation. Response

rates have remained high and only 511 participants have actively denied to continue over the years.

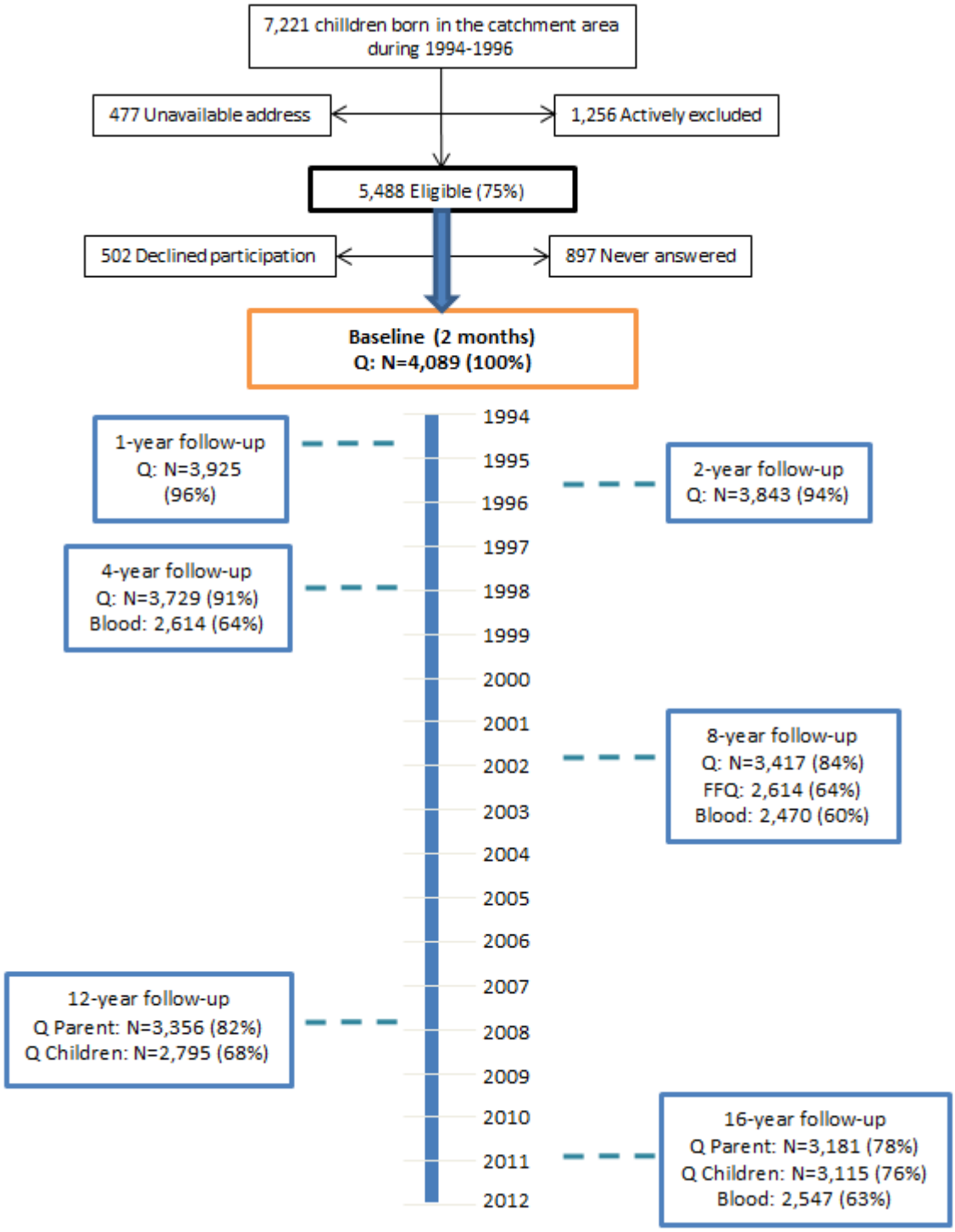

Figure 4.2 Flow chart of the BAMSE cohort. The initial inclusion and the follow-ups over time are described. Q=Questionnaire. At the 12 and 16 year follow-ups parents and children received separate questionnaires. Blood=Blood sample obtained at the clinical examination.

4.2 EXPOSURE AND COVARIATE ASSESSMENT

4.2.1 Questionnaire-based data

Information on fish intake and most covariates assessed as confounders was derived from questionnaires. In **study II** we used information on current frequency of fish intake from the 1-year questionnaire, in which the parents had five predefined response categories to choose from (**Figure 4.3**). A food frequency questionnaire (FFQ) was filled out at the 8-year clinical examination. The FFQ included questions on average frequency of consumption during the past year of 103 food and beverage items commonly consumed in Sweden. The ten predefined response categories ranged from never to three or more times per day. Forty percent of the FFQs were answered by a parent together with the child and 57% were answered by parent only; in total 2,614 FFQs were filled out. In one block of questions the frequency of consumption of fish species was asked for (**Figure 4.4**). We used information on fish consumption from the FFQ in **studies II, III** and **IV**.

47. How often does the child eat fish?

- ☐ More than once a week
- ☐ Once a week
- ☐ 2-3 times a month
- ☐ Once a month or less
- ☐ Never eats fish

Figure 4.3 The question regarding fish consumption included in the 1-year questionnaire.

How often has your child, on average, consumed the following food items at home and in school during the past 12 months?

FOOD ITEMS, FOOD COURSES	Never	Less than 1 time per month	1-3 times per month	Times per week				Times per day		
				1	2	3-4	5-6	1	2	3+
FISH										
Herring, mackerel	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Salmon	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cod, pollock, pike	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fish fingers	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Caviar (e.g., Kalles)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tunafish	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Shellfish	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Figure 4.4 Section on fish consumption from the food frequency questionnaire filled out at age 8 years. The section has been edited and translated from Swedish.

The responses to the FFQ were calculated for individual energy and nutrient intake with the use of food composition values from the database at the Swedish National Food Agency.[132] In a first step, the intake frequency of each food item in the FFQ was multiplied with its nutrient content per portion. If an exact portion size was not stated in the FFQ, portion sizes based on the consumption in the national survey Riksmaten 2003 were used instead. Riksmaten 2003 is based on a representative sample of 8-9-year old children in Sweden.[133] Children in Riksmaten registered their food consumption in a food diary during four consecutive days. In a second step, nutrient values were summarized over foods and beverages to obtain calculated total intake of each nutrient. To remove the variation in nutrient intake caused by differences in total energy consumption, the calculated nutrient levels were energy-adjusted using the residual method suggested by Willet et al.[134] After energy-adjustment with the residual method, the remaining variation was caused by differences in nutrient composition. Children with an implausible energy intake were excluded (±3 log SDs; ≤842.5 or ≥3961.1 kcal per day). In **studies III** and **IV** we used intakes of polyunsaturated fatty acids (PUFAs) calculated from the FFQ. In addition, in **study III** we used the calculated intake of vitamin D.

The FFQ-based data on fish and PUFA intake has some limitations. FFQs are generally considered to give rise to some misclassification, since they often ask about consumption for a long time frame, for example, the preceding year. Exact portion sizes are not usually inquired about, but rather frequency of intake, which is then used to calculate nutrient intake in grams per day. FFQs are useful tools to distinguish subjects into different categories based on amount of intake. FFQs are often used in epidemiological studies, as they are cheap and easy to distribute among many participants.

4.2.2 Analysis of plasma fatty acid composition

For **study IV** the composition of fatty acids in plasma phospholipids was measured in blood samples collected at the 8-year clinical examination (**Figure 4.2**). For the plasma fatty acid analysis, 999 children were considered available. In pilot measurements, 60 of these were used to see if the method would work in our study population; if there were measureable amounts of fatty acids in the samples and if there was a range of exposure (fatty acids) for this group of children. In the end, 940 children had plasma composition measured, while 59 had too small amounts of blood left in the sample (**Figure 4.5**). The fatty acid composition of plasma phospholipids was measured using gas chromatography. Fifteen different fatty acids were detected. Each fatty acid was then measured as the relative amount of all fatty acids in the sample. This method is often used instead of measuring absolute amounts, since absolute amounts are more complicated, more time-consuming and more expensive to measure. The correlation between relative and absolute amounts has been shown to be good for most fatty acids.[135] One sample had an undetectable amount of α-linolenic acid (ALA, 18:3 n-3); this child was included in the statistical analyses of the other fatty acids.

The blood samples had been stored for just over ten years before the fatty acid content was analyzed. The samples were frozen in minus 80ºC, a temperature at which the fatty acids should be stable.[136] If degradation has occurred it should affect all samples equally and therefore not introduce any misclassification bias.

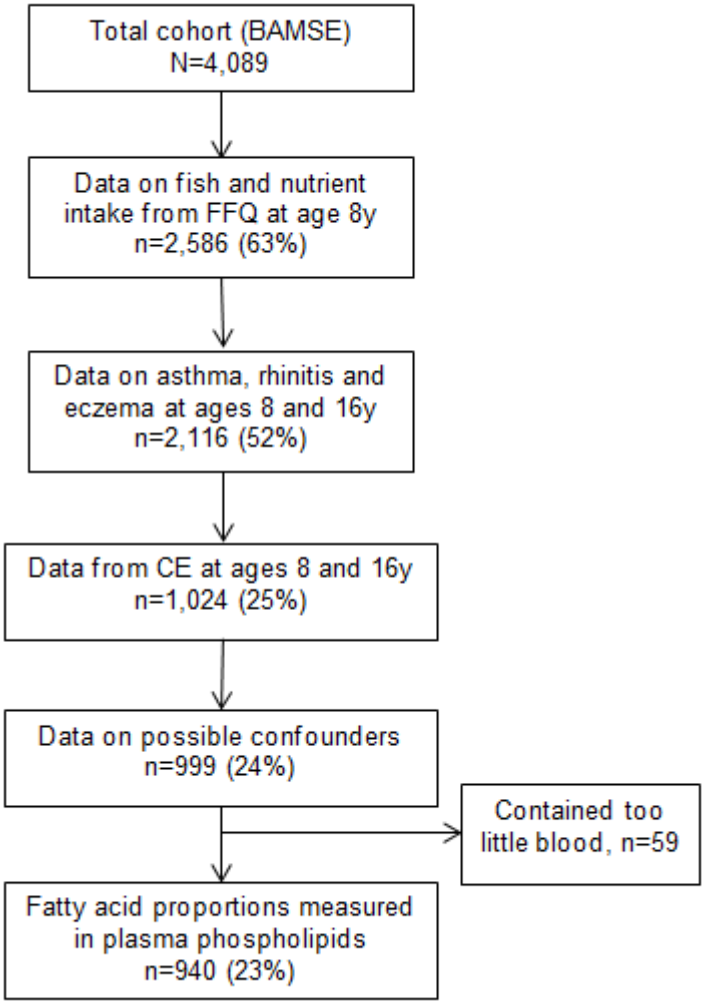

Figure 4.5 Inclusion of children to the plasma fatty acid analysis. FFQ=Food frequency questionnaire filled out at the 8-year clinical examination, CE=Clinical examination.

4.2.3 Collection of weight and height data from school health care

To be able to study overweight status over time in BAMSE we asked for permission in the 12-year questionnaire to collect weight and height data from child- and school health center registers. We received consent to collect data for 3,151 children. Children in Sweden are measured and weighed regularly at child welfare centers between ages 0 and 6 years, after which their charts are transferred with them to school. Through the school health service they are measured and weighed when they start school and then in the second, fourth, sixth and eighth grade. The first step in the collection process was to find out about the children's schools. To gain this information I turned to the municipalities where they lived. In all, 149

municipalities were contacted and 137 gave me the information I needed. The children attended 447 different schools. The second step was to contact the school nurses. For schools with over ten children from BAMSE in attendance, I or a colleague visited and collected the data. For schools with fewer children, we sent a letter with information and a data sheet to fill out for each child. In total, we obtained data for 2,597 children for up to ten different ages (**Table 4.1**). The amount of available data varied across the ages.

Table 4.1 Weight and height data collected from school health centers for a sub-population of the BAMSE cohort[1]

Source	Pre-defined age (y)	Time span	n	% of cohort
Child health center	0.5	±2 w	2,317	57
	1	±4 w	2,302	56
	1.5		2,259	55
	2	±6 mo	1,610	39
	3		1,301	32
	4		2,271	56
	5		2,214	54
School health center	7	-6 to + 11 mo	2,473	60
	10		2,252	55
	12		2,267	55

1. Data available from a sample of those answering the 12-year questionnaire

We collected weight and height data retrospectively for the subjects who gave their consent in the 12-year questionnaire. This method might have introduced a selection bias, if participants gave their consent based on their weight (see more about selection bias in section 7.1.2.1). For children who were weighed and got their height measured at the 8-year clinical examination we could check if that was the case. There was no difference in the proportion of consent among the children who were considered overweight (isoBMI≥25 kg/m²) at age 8 years compared with those who were considered normal weight (isoBMI<25 kg/m²) (*P*-value from chi^2-test: 0.068). Regarding obesity status there was a significant difference, with fewer obese (isoBMI≥30 kg/m²) than expected among the ones who gave consent (*P*-value from chi^2: 0.002). However, the obesity prevalence for the sub-sample of children with data from the collection is comparable with the prevalence for the cohort at age 8 years (3.9% [95% CI: 3.1-4.7] vs. 4.4% [95% CI: 3.6-5.2]). Overall, we should be cautious to generalize our result to an obese population; there were few children classified as obese overall and I did not study this group separately.

4.3 ALLERGIC DISEASE AND SENSITIZATION ASSESSMENT

The information used to define allergic disease was retrieved from the questionnaires distributed during childhood. We relied on parental report of symptoms for children up to 12 years of age. At 16 years of age the children themselves answered a questionnaire which we used to define disease. Definitions of allergic disease are described in **Table 4.2**.

Table 4.2 Definitions of allergic disease

Variable	Definition	Study
Asthma at ages 1 & 2y	At least three episodes of wheeze in combination with inhaled steroids or signs of bronchial hyperreactivity without concurrent upper respiratory infection	**II**
Asthma at ages 4, 8, 12 & 16y	At least four episodes of wheeze in the previous 12 months or at least one episode of wheeze during the same period in combination with occasional or regular use of prescribed inhaled steroids	**I** (8y), **II** & **IV** (8 & 16y)
Rhinitis at ages 1 & 2y	Prolonged rhinitis symptoms, like a runny or blocked nose, for at least two months in the past 12 months	**II**
Rhinitis at ages 4, 8, 12 & 16y	Prolonged rhinitis symptoms without a common cold in the past 12 months	**II**
Rhinitis at ages 8 & 16y	Prolonged rhinitis symptoms without a common cold in the past 12 months and/or symptoms following contact with furred pets, pollens, or mites	**III** (16y) & **IV** (8 & 16y)
Eczema at ages 1 & 2y	Dry skin in combination with an itchy rash at a typical location (face, or arm/leg extension surfaces, or arm/leg flexures, or wrist/ankle) for at least two weeks during the past 12 months OR A doctor's diagnosis of eczema during the past 12 months	**II**
Eczema at age 4y	Dry skin in combination with an itchy rash at a typical location (face, or arm/leg extension surfaces, or arm/leg flexures, or wrist/ankle) for at least two weeks during the past 12 months OR Usage of cortisone ointment during the past 12 months	**II**
Eczema at age 8y	Dry skin in combination with an itchy rash at a typical location (face, or arm/leg flexures, or wrist/ankle, or neck) for at least two weeks during the past 12 months OR A doctor's diagnosis of eczema during the past 12 months	**II**
Eczema at age 12y	Dry skin in combination with an itchy rash at a typical location (face, or arm/leg flexures, or wrist/ankle, or neck) for at least two weeks during the past 12 months	**II**

4.3.1 Sensitization

Allergic sensitization was measured in blood samples provided at the three clinical examinations. Immunoglobulin E (IgE) antibodies in blood were analyzed with ImmunoCAP to common aeroallergens by using the Phadiatop® mix (cat, dog and horse dander; timothy, birch and mugwort pollen; *Cladosporium herbarum* (fungi) and *Dermatophagoides pteronyssinus* (house dust mite); Thermo Fisher Specific, Uppsala, Sweden). A sample positive for the Phadiatop mix was analyzed further for allergen-specific IgE antibodies to the allergens listed above, and in addition to *Dermatophagoides farinae* (house dust mite) at age 16 years. Moreover, IgE antibodies to common food allergens were also analyzed using the fx5® mix (cow's milk, hen's egg, codfish, soybean, peanut, and wheat). A technical cut-off for a positive test was set at 0.35 kU/L, in accordance with the manufacturer's instructions.

Sensitization status was used as a separate outcome and to divide the diseases into IgE-associated and non-IgE-associated phenotypes.

4.4 STUDY DESIGN & POPULATION

All studies included in this thesis have an observational design, meaning that we as researchers collect data and study associations of exposure and outcome without interfering with the exposure. As mentioned before, the studies are based on the birth cohort BAMSE.

4.4.1 Study I

The study included 2,075 children for whom there was information available on weight and height at ages 1 and 7 years and on asthma at age 8 years (see **Table 4.5** for more details). **Study I** was a longitudinal study, meaning that the occasions of exposure and outcome were separated in time, and that exposure occurred before the outcome. Longitudinal studies can be divided into prospective and retrospective studies. In prospective studies the exposure is assessed before the outcome has occurred. In contrast, in retrospective studies exposure information is gathered after the outcome has occurred. Although, the information on weight and height used in **Study I** was collected when the children were around 12 years of age, the measurements of weight and height were done when the children were 1-7 years of age. A potential issue regarding the weight and height data is selection bias; this is discussed further in the discussion section of this thesis.

4.4.2 Study II

For this study, 3,285 children were included, for whom there was information available on fish consumption at age 1 year and on at least one outcome at age 12 years (see **Table 4.5** for more details). The study was longitudinal and prospective, the exposure was retrieved from the 1-year follow-up and information on asthma, rhinitis and eczema status was obtained from all follow-ups from ages 1 to 12 years (**Figure 4.2**). The repeated measurements of outcome over time in the same individual are a special case of longitudinal design which introduces the need for different statistical methods that can take care of the intra-correlation between observations. In addition, sensitization status was used from the 8-year follow-up.

4.4.3 Study III

The study included 1,970 children who had provided information on fish and nutrient intake at age 8 years and on outcome data at age 16 years (see **Table 4.5** for more details). The study was longitudinal and prospective. In addition, cross-sectional association analyses between fish intake at age 8 years and outcome at the same age were performed, for which the data on exposure and outcome was collected at the same point in time. This kind of

analysis gives information of possible associations, but is not suitable for drawing conclusions about temporal relationships.

4.4.4 Study IV

The study was longitudinal and prospective and consisted of the 940 children for whom there was information on fatty acid proportions in plasma at age 8 years and outcome data at age 16 years (**Figure 4.5** and see **Table 4.5** for more details). In addition, cross-sectional association analyses between fatty acid proportions at age 8 years and outcome at age 8 years were performed.

4.5 STATISTICS

One-sample *t*-test was used to compare the distribution of selected background characteristics in the baseline cohort with the specific study populations included in each study.

4.5.1 Exposure modelling

The exposures used in the different studies are defined in **Table 4.3**. The lowest category of exposure was always set as the reference. For test of trends in association analyses of nutrient variables in **study III**, the median intake level for each tertile was assigned to everyone in that tertile and used as a continuous variable.

Table 4.3 Modelling of exposures

Variable	Definition	Study
BMI at ages 1, 1.5, 4 & 7y	Body weight in kilograms divided by height in meters squared (kg/m²). Normal BMI vs. high BMI (≥85th percentile[1])	**I**
Change in BMI status between early age (12/18 months) & 7y, and between 4 & 7y	• Persistent normal BMI (normal BMI at both early age & at 7y) • Early high BMI (high BMI at early age but normal BMI at age 7y) • Late high BMI (normal BMI at early age but high BMI at age 7y) • Persistent high BMI (high BMI both at early age & at age 7y) Classification of change between 4 & 7 years was done in the same way	**I**
At 1y, intake of:		
Fish	Categorical variable (see **fig 4.3**) and dichotomous variable (≤1 time/month (irregular) vs. ≥2-3 times/month (regular))	**II**
At 8 y, intake of:		
Total fish	Sum of herring/mackerel, salmon fishes, cod/pollock/pike, fish fingers & tuna fish (<2 times/week vs. ≥2 times/week)	**III**
Oily fish	Sum of herring/mackerel and salmon fishes (<1 time/week vs. ≥1 time/week)	**III**
Cod/pollock/pike	<1 time/week vs. ≥1 time/week	**III**
Fish fingers	<1 time/week vs. ≥1 time/week	**III**
Total PUFA	Sum of LA, ALA, AA, EPA, DPA & DHA[2] (Tertiles)	**III**
Total VLC n-3	Sum of EPA, DPA & DHA (Tertiles)	**III**
Total n-6	Sum of LA & AA (Tertiles)	**III**
n-6/n-3	The ratio of total n-6 to total n-3 (Tertiles)	**III**
LA, ALA, AA	Used as separate exposures (Tertiles)	**III**
Vitamin D	Tertiles	**III**
Plasma levels of:		
Total VLC n-3	Sum of EPA, DPA & DHA (Continuous)	**IV**
LA, ALA, AA	Used as separate exposures. ALA was log-transformed to improve normality (Continuous)	**IV**

1. Based on children in the study
2. LA=Linoleic acid (18:2 n-6); ALA=α-Linolenic acid (18:3 n-3); AA=Arachidonic acid (20:4 n-6); EPA=Eicosapentaeonic acid (20:5 n-3); DPA=Docosapentaeonic acid (22:5 n-3); DHA=Docosahexaeonic acid (22:6 n-3)

4.5.2 Outcome modelling

See **Table 4.5** for an overview of the outcomes for the different studies.

Asthma, eczema and rhinitis were dichotomized as no vs. yes based on the disease definition (see **Table 4.2** for the definitions). For sensitization IgE≥0.35 kU/L was set as the cut-off for a positive test. In **study I**, sensitization to aeroallergens and food allergens was analyzed separately, while it was analyzed as total sensitization in **study II**. In **study IV**, sensitization to aeroallergens was defined as having a positive test to the Phadiatop® mix plus at least one specific aeroallergen.

For analyses of phenotypes of allergic disease the subjects were divided into categories;

- No specific allergic disease and no sensitization (reference category)
- Specific allergic disease but no sensitization (non-IgE associated phenotype)

- Specific allergic disease and sensitization (IgE-associated phenotype)

For **study II**, total sensitization (Phadiatop® and/or fx5®) was used, while Phadiatop® was used to discriminate between IgE-associated and non-IgE-associated phenotypes in **study III**. In **study IV**, Phadiatop® in addition to at least one positive test to a specific aeroallergen was used to discriminate between phenotypes.

Incidence of disease was examined in **studies II**, **III** and **IV**. In **study II**, incidence was defined as fulfilling the definition of disease at the given age, but not at any previous follow-up. In **study III**, children with rhinitis symptoms at 8 years of age were excluded to study incidence of IgE-associated (allergic) and non-IgE-associated (non-allergic) rhinitis up to age 16 years. In **study IV**, incidence was defined as fulfilling the definition of disease at age 16 years, but not at ages 4, 8 or both.

4.5.3 Statistical analyses

All statistical analyses were performed with Stata 11 software (StataCorp, College Station, Tx). *P* values below 0.05 were considered statistically significant. This is an arbitrary cut-off, often used in epidemiological studies.

I used logistic regression to assess potential associations between exposures and dichotomous outcomes of allergic disease in all studies. To examine the potential associations between exposures and IgE-associated and non-IgE-associated phenotypes, I applied multinomial logistic regression in **studies II**, **III** and **IV**. Moreover, in **study II**, I used Generalized Estimating Equations (GEE) because of the use of repeated outcome measurements for the subjects. GEE takes into account within-person correlation and provides estimates when missing observations are unequally spaced.[137] The model incorporates an interaction between time and exposure to evaluate the effect of exposure over time. The results from the regression models and GEE are presented as odds ratios (ORs) with 95% confidence intervals (95% CI).

A range of potential confounders were tested by checking each factor's impact in the crude model of the exposure and the outcome in each study. The ones finally included in the multivariable analyses for the different studies are described in **Table 4.4** and listed in **Table 4.5**. Potential confounders to test for were chosen based on a priori knowledge. For a factor to be a confounder it needs to be associated with the exposure, have an independent effect on the outcome, and should not be affected by the exposure or the disease. A factor that is in the causal chain between exposure and outcome is a mediator and can be adjusted for only if you would like to study the direct effect of the exposure on the outcome, instead of the total effect. This was tested in **study II**, where I adjusted for childhood diet at age 8 years (fish, vitamin D, total energy and use of dietary supplements) in the analyses of infant fish consumption and allergic disease throughout childhood; however, additional adjustment did not affect the observed results. Another way to deal with confounding is to stratify the

analyses by the potential confounding factor. In **studies I** and **II,** I stratified by parental allergic disease.

Table 4.4 Definition and modelling of confounders

Variable	Definition	Study
Sex	Female vs. male	**I**, **II** & **IV**
Parental allergic disease	Mother and/or father with a doctor's diagnosis of asthma & asthma medication and/or a doctor's diagnosis of hay fever in combination with furred pet and/or pollen allergy (no vs. yes)	**I**, **II** & **IV**
Maternal smoking	Mother smoked at least one cigarette per day at any time point during pregnancy or when the child was an infant (no vs. yes)	**I**, **II**
Maternal age	Maternal age at birth of the child (>25y vs. ≤25y)	**I**
Birth weight	In grams (tertiles)	**I**
Breastfeeding duration	Exclusive breastfeeding without exposure to formula (cow's milk or hydrolysate) or solid foods (>4 month vs. ≤4 month)	**I**
Total intake of carbohydrates	g/day (continuous)	**III**
Total intake of monounsaturated fat	g/day (continuous)	**III**

A potential problem that can affect observed ORs is disease-related modification of exposure. This is typically not a problem in longitudinal studies because the exposure is measured before the disease of interest. However, disease-related modification of exposure can be problematic in the case of allergic disease, because many children have transient asthma-like symptoms and dry skin during the first years of life or might have allergic parents, characteristics which might lead to changes of their dietary habits, for example their fish consumption. This particular problem was addressed through different sensitivity analyses in the different studies. In **studies I**, **III** and **IV** additional adjustment for early symptoms of allergic disease, defined as reported symptoms of wheeze, eczema or both during the first 2 years of life, was performed. In addition, in **study I**, children with reported wheeze between the ages 3 months and 1 year and with reported doctor's diagnosis of asthma during the first 2 years of life were excluded from secondary analyses. Children with symptoms of wheeze, eczema or both during the first year of life were excluded in **study II**. Finally, interactions between exposures and parental allergic disease were tested with Wald test or Likelihood ratio test in **studies II**, **III** and **IV**.

Table 4.5 Overview of the studies

	Study I	Study II	Study III	Study IV
Follow-ups used	Weight & height from SHC[1] (permission in Q[2]12), Q0-8, CE[3]8	Q0-12, CE8, FFQ8[4]	Q0, Q1, Q2, Q8, Q16, Qc16[5], FFQ8, CE16	Q0, Q1, Q2, Q8, Qc16, FFQ8, CE4-CE16, plasma FA data[6]
N, % of the cohort	2,075, 51%	3,285, 80%	1,970, 48%	940, 23%
Inclusion criteria	Q0-8, weight & height at 1 & 7y, asthma at 8y	Q0, fish intake at 1y, ≥1 outcome at 12y	Q0, nutrient & total fish intake at 8y, outcome data at 16y	Q0, plasma FA, outcome data at 16y
Exposures	BMI[7] at age 1, 1.5, 4 & 7y	Infant fish intake	Fish & PUFAs intake at school age	Plasma PUFAs at age 8y
Outcomes	Prevalent asthma & sensitization 8y	Prevalent & incident asthma, rhinitis & eczema 1-12y	Incident rhinitis between 8 & 16 y	Prevalent & incident asthma, rhinitis & aeroallergen sensitization 8 & 16y
Allergic and non-allergic phenotypes		8y	16y	16y
Confounders adjusted for	Sex, parental allergic disease, maternal smoking, maternal age, birth weight, breastfeeding duration	Sex, parental allergic disease, maternal smoking	Fish analyses adj for the other types of fish. PUFA & vit D analyses adj for total intake of carbohydrates, monounsaturated fat & the other exposures	Sex, parental allergic disease
Statistical methods for association analyses	Multivariable log reg.	GEE. Multivariable log reg. Multinomial log reg.	Multinomial log reg.	Multivariable log reg. Multinomial log reg. Spearman rank correlation test

1. SHC=School health center
2. Q=Questionnaire at age 1 to 16 years
3. CE=Clinical examination at age 4, 8 & 16 years
4. FFQ=Food frequency questionnaire at age 8 years
5. Qc=Questionnaire answered by the children themselves
6. Plasma FA data=FA proportions measured in plasma phospholipids
7. BMI=Body mass index (kg/m^2)

5 ETHICAL APPROVALS

All participants in BAMSE have given their informed consent. All have been informed that they have the right to withdraw their participation in each follow-up. All follow-ups of the BAMSE study were approved by the ethical review board at Karolinska Institutet, Stockholm, Swede

6 RESULTS

6.1 BODY MASS INDEX IN RELATION TO ASTHMA AND ALLERGIC SENSITIZATION AT AGE 8 YEARS

The distribution of background characteristics for the 2,075 children included in **study I** (the inclusion criteria are found in **Table 4.5**) was comparable to the baseline cohort. The prevalence of high body mass index (BMI) ranged between 14 and 15% at ages 1 year, 1.5 years, 4 years and 7 years. Children with a high BMI at an early age (1 year and/or 1.5 years) were more likely to have been exposed to maternal smoking during pregnancy and/or infancy and were in general heavier at birth (see **Table 1** in **study I**). At age 8 years, a total of 124 children fulfilled the definition of asthma, 402 children were sensitized to aeroallergens and 310 children to food allergens.

A high BMI at ages 1, 4 and 7 years was associated with an increased risk of asthma at age 8 years (OR_{adj}: 1.62 [95% CI: 1.01-2.60] for high BMI at age 1 year, OR_{adj}: 1.72 [95% CI: 1.06-2.80] for high BMI at age 4 years, and OR_{adj}: 2.13 [95% CI: 1.36-3.35] for high BMI at age 7 years). When we tried to disentangle the timing of high BMI, by dividing the children into different categories based on their BMI status at each age, we observed that children who had a high BMI at early age or 4 years but a normalized BMI at age 7 years had no increased risk of asthma at age 8 years (**Figure 6.1**). In contrast, there was a significant association between late high BMI and an increased risk of asthma at age 8 years. In addition, a persistent high BMI between ages 4 and 7 years was associated with an increased risk of asthma at age 8 years.

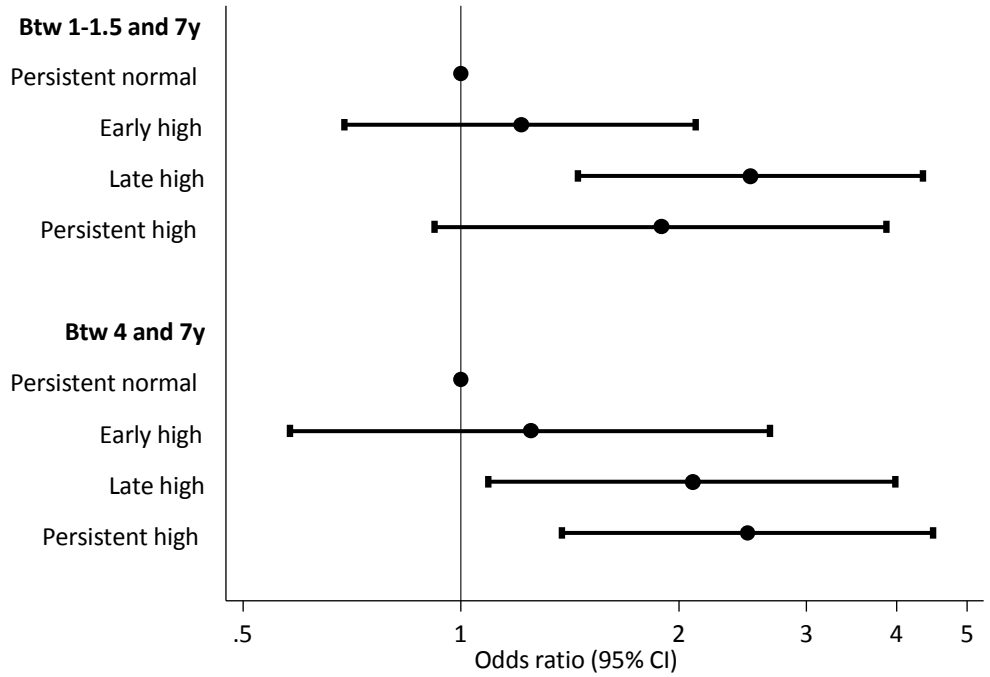

Figure 6.1 Association between change in BMI status and asthma at age 8 years in multivariable analyses adjusted for sex, parental allergic disease, maternal smoking, maternal age, birth weight, and breastfeeding duration.

To address the issue of a possible effect of early symptoms of allergic disease on BMI, the analyses were additionally adjusted for early symptoms of allergic disease (wheeze and/or eczema during the first 2 years of life). The additional adjustment did not affect the observed associations. We also excluded children with reported wheeze during the first year of life and a reported doctor's diagnosis of asthma during the first 2 years of life. Among the 1,698 children in the analyses after exclusion, a high BMI at age 7 years was still associated with asthma at age 8 years (OR_{adj}: 2.48 [95% CI: 1.40-4.38]), whereas a high BMI at earlier ages was not.

High BMI at age 1 and 7 years, respectively, was associated with an increased risk of sensitization to aeroallergens at age 8 years (OR_{adj}: 1.43 [95% CI: 1.04-1.96] for high BMI at age 1 year, and OR_{adj}: 1.42 [95% CI: 1.02-1.97] for high BMI at age 7 years). In analyses of change in BMI status, early high BMI was not associated with aeroallergen sensitization at age 8 years, while children who had developed a high BMI between early age and 7 years had an increased risk of being sensitized to aeroallergens (OR_{adj}: 1.59 [95% CI:1.06-2.39]). We did not observe any significant associations between high BMI and food sensitization (data not shown).

6.2 FISH CONSUMPTION IN RELATION TO ALLERGIC DISEASE

6.2.1 Infant fish consumption and allergic disease up to age 12 years

The 3,285 children included **in study II** (inclusion criteria are found in **Table 4.5**) were comparable to the children in the baseline cohort, except that they had a somewhat higher prevalence of white collar workers among their parents (83.2% vs. 81.6%) (see **Table 1** in **study II**). Eighty percent of the children had a regular consumption of fish (2 times/month or more) at age 1 year. Parental allergic disease and early symptoms of allergic disease (wheeze and/or eczema during the first year of life) delayed the introduction of fish and led to lower intake at age 1 year ($P<0.001$). The prevalence of asthma was stable from age 1 to 12 years (see **Table 2** in **study II**). Rhinitis prevalence increased during school ages, while the prevalence of eczema decreased during the same time period. The incidence of the different diseases followed the same patterns. At age 12 years, 218 children fulfilled the definition of asthma, 681 fulfilled the definition of rhinitis and 392 fulfilled the definition of eczema.

The associations between fish intake (per category) at age 1 year and overall risk of asthma, rhinitis and eczema (1-12 years) are displayed in **Figure 6.2**. Increased fish intake at age 1 year was inversely associated with overall reduced risk of asthma (P trend<0.001), rhinitis (P trend<0.001) and eczema (P trend<0.001). Restriction to children without early symptoms of allergic disease attenuated the observed associations; the inverse association with rhinitis was the only one still significant (OR_{adj}: 0.63 [95% CI: 0.46-0.87] comparing the highest with the lowest intake, P trend<0.001).

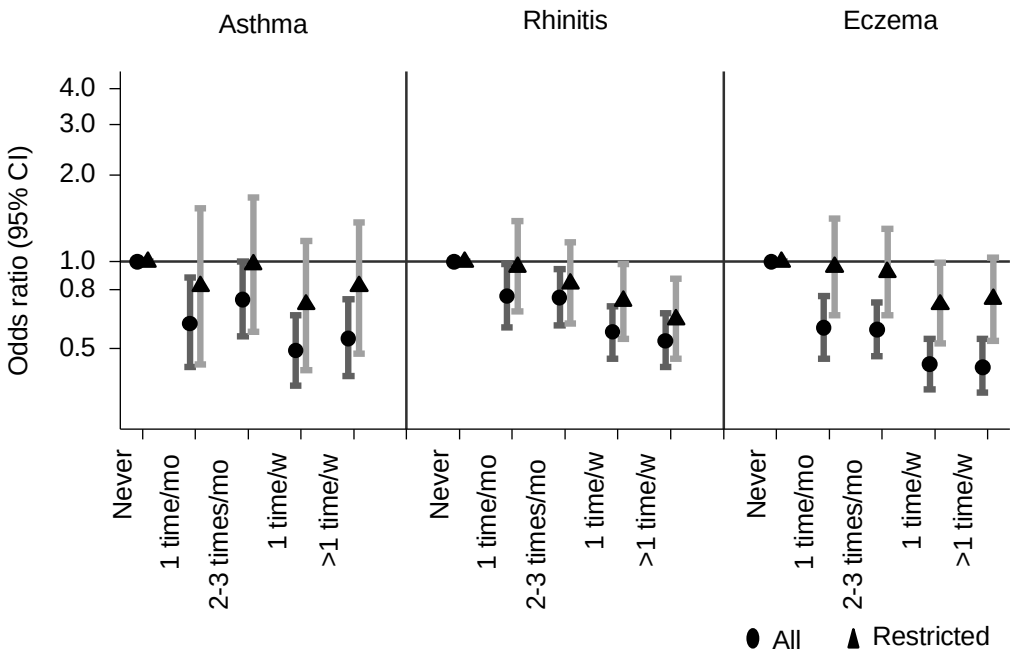

Figure 6.2 Associations between categorical fish intake at age 1 year and overall risk of asthma, rhinitis and eczema (1-12 years) in multivariable GEE analyses adjusted for sex, parental allergic disease, and maternal smoking. The associations are displayed for all children (circles) and after restriction to children without symptoms of allergic disease during the first year of life (triangles).

Analyses of dichotomized fish consumption revealed reduced risks of asthma, rhinitis and eczema over all time points (1-12 years) for children with regular intake (≥2-3 times/month) compared with children with irregular intake (≤1 time/month) (see **Figures 1**, **2** and **3** in **study II**). The estimates for eczema became gradually closer to one and were non-significant at age 12 years in contrast to the estimates for asthma and rhinitis, which were more stable over time. After restricting the analyses to children without early symptoms of allergic disease, regular fish intake at age 1 year was associated with a reduced risk of overall rhinitis (OR_{adj}: 0.74 [95% CI: 0.60-0.90]) and eczema (OR_{adj}: 0.78 [95% CI: 0.63-0.97]).

The estimates for incidence of asthma, rhinitis and eczema were similar overall to the estimates for prevalent asthma, rhinitis and eczema (see **Figures 1**, **2** and **3** in **study II**). After restriction of the analyses to children without early symptoms of allergic disease regular fish intake at age 1 year was still associated with a reduced risk of incident rhinitis (OR_{adj}: 0.78 [95% CI: 0.63-0.95]).

Regular fish intake at age 1 year was associated with a reduced risk of aeroallergen and/or food sensitization at age 8 years (OR_{adj}: 0.78 [95% CI: 0.63-0.96]). After restriction to children without early symptoms of allergic disease the association vanished (OR_{adj}: 0.98 [95% CI: 0.73-1.30]).

In analyses of IgE-associated and non-IgE-associated disease at age 8 years, the only significant association, after restriction to children without early symptoms of allergic

disease, was observed for non-IgE-associated rhinitis (OR_{adj}: 0.36 [95% CI: 0.21-0.63]) (**Figure 6.3**).

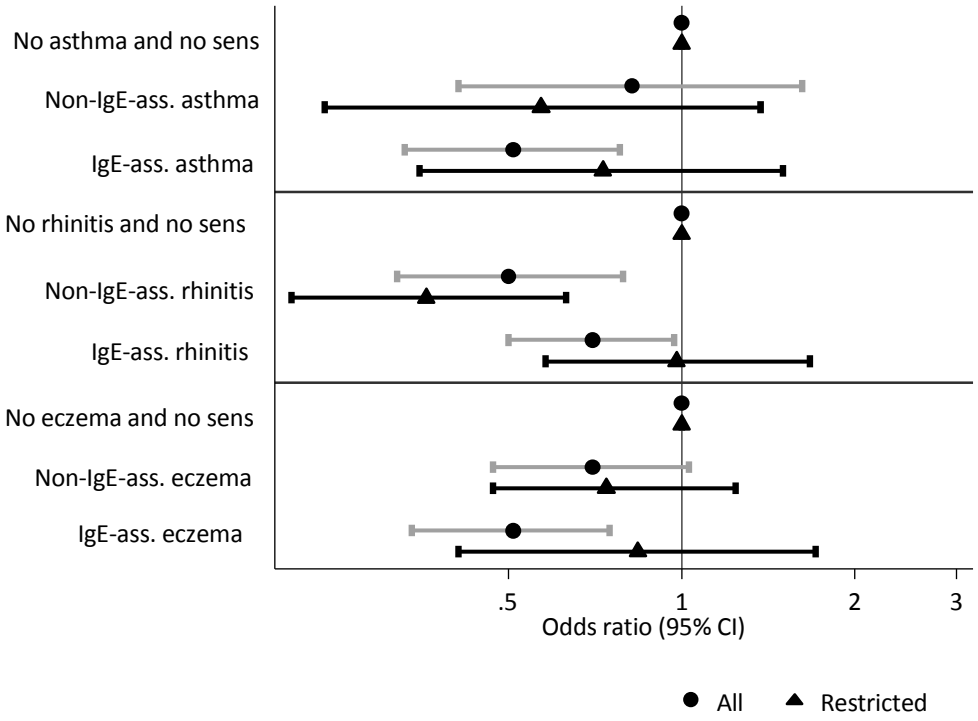

Figure 6.3 Associations between a regular fish intake at age 1 year (≥2-3 times/month) and non-IgE-associated and IgE-associated phenotypes of asthma, rhinitis and eczema at age 8 years, in multivariable analyses adjusted for sex, parental allergic disease, and maternal smoking. The associations are displayed for all children (circles) and after restriction to children without symptoms of allergic disease during the first year of life (triangles).

6.2.2 Childhood fish consumption and development of rhinitis

The distribution of characteristics for the 1,970 children included in **study III** (inclusion criteria are found in **Table 4.5**) was comparable to the children in the baseline cohort; only minor differences were observed (see **Table EI** in the online repository of **study III**). The median intake of fish at age 8 years was 1.7 times/week and 38% consumed fish two times per week or more. Fourteen percent consumed oily fish once per week or more, while cod/pollock/pike and fish fingers were consumed regularly by 32% and 38%, respectively. The cumulative incidence of IgE-associated rhinitis (AR) and non-IgE-associated rhinitis (NAR) between ages 8 and 16 years was 21% (n=337) and 15% (n=236), respectively. Other characteristics of the study population are found in **Table I** in **study III.**

Total fish intake at age 8 years was not associated with incidence of AR or NAR between ages 8 and 16 years. In analyses of types of fish we observed that regular intake of oily fish was associated with a reduced risk of incident NAR (OR_{adj}: 0.52 [95% CI: 0.32-0.87]). The association with AR was in the same direction but did not reach statistical significance (OR_{adj}: 0.78 [95% CI: 0.53-1.15]). No clear associations were observed between

cod/pollock/pike or fish fingers and AR or NAR (**Figure 6.4**). Fish intake at age 8 years was not associated with AR or NAR at age 8 years.

Additional adjustment for early symptoms of allergic disease (wheeze and/or eczema during the first 2 years of life) did not affect the observed associations between oily fish and incident NAR (OR_{adj}: 0.48 [95% CI: 0.28-0.82]).

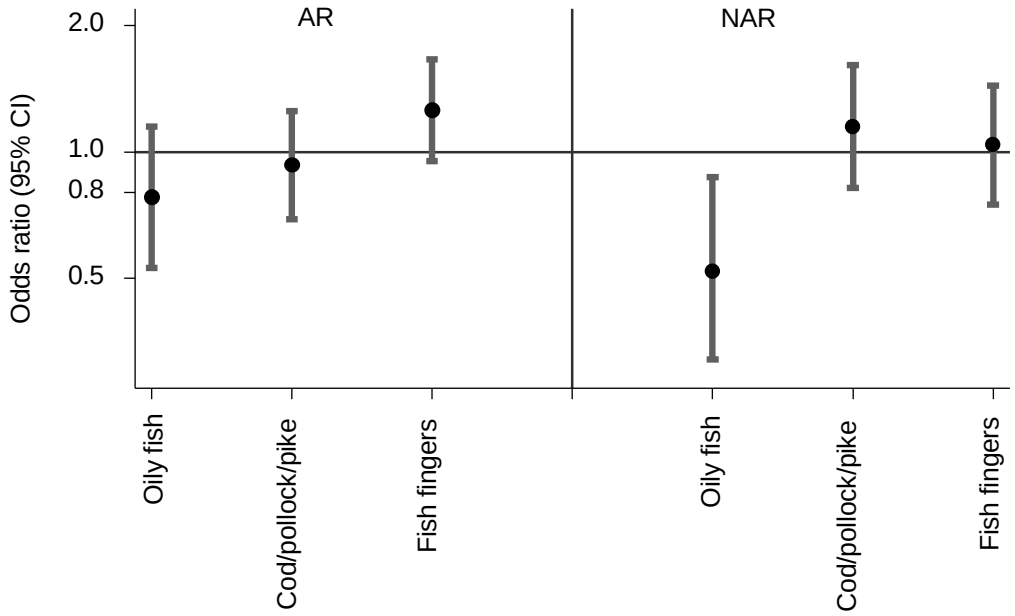

Figure 6.4 Association between intake of different types of fish at age 8 years and incidence of AR and NAR between ages 8 and 16 years. The association for each type of fish was adjusted for the other types of fish.

Since the results for AR and NAR went in the same direction, cases of AR and NAR were combined in post hoc analyses to raise the comparability with previously published results. Oily fish was associated with a reduced risk of incident rhinitis between ages 8 and 16 years (OR_{adj}: 0.67 [95% CI: 0.48-0.97]).

6.3 POLYUNSATURATED FATTY ACIDS IN RELATION TO ALLERGIC DISEASE

6.3.1 Polyunsaturated fatty acid intake and development of rhinitis

The median intake of total polyunsaturated fatty acids (PUFAs), total n-6 fatty acids (the sum of linoleic acid [LA, 18:2] and arachidonic acid [AA, 20:4]) and total VLC n-3 fatty acids (the sum of eicosapentaeonic acid [EPA, 20:5], docosapentaeonic acid [DPA, 22:5], and docosahexaeonic acid [DHA, 22:6]) for the 1,970 children in **study III** were 7.9 g/day, 6.2 g/day and 0.2 g/day, respectively (**Table 6.1**). Salmon was the major source for intake of total VLC n-3 fatty acids, while the dietary sources for total n-6 fatty acids were more diverse (**Figure 6.5**). The cumulative incidence of AR and NAR between ages 8 and 16 years were 21% (n=337) and 15% (n=236), respectively.

Table 6.1 Consumption of PUFAs[1] and vitamin D at age 8 years, for the study population (N=1,970), and for adolescents with incidence of IgE-associated rhinitis (AR, n=337) or non-IgE-associated rhinitis (NAR, n=236) between ages 8 and 16 years

	Study population		Incident AR		Incident NAR	
PUFA (g/day)	**Median**	**IQR[2]**	**Median**	**IQR[2]**	**Median**	**IQR[2]**
Total PUFA	7.9	9.6-6.5	7.9	9.6-6.6	8.0	9.7-6.4
ALA[3]	1.1	1.4-0.9	1.1	1.4-0.9	1.2	1.5-0.9
VLC n-3[4]	0.2	0.3-0.1	0.2	0.3-0.2	0.2	0.3-0.1
n-6[5]	6.2	7.6-5.2	6.3	7.6-5.2	6.2	7.9-5.2
LA	6.2	7.5-5.1	6.2	7.5-5.2	6.2	7.7-5.1
AA	0.1	0.09-0.06	0.1	0.09-0.06	0.1	0.09-0.05
n-6/n-3 ratio	4.6	4.9-4.2	4.6	4.9-4.2	4.7	5.0-4.3
Vitamin D (µg/day)	5.1	6.5-3.9	5.1	6.4-3.9	5.3	6.4-3.9

1. PUFAs=Polyunsaturated fatty acids
2. IQR=Interquartile range
3. ALA=α-linolenic acid (18:3 n-3)
4. Sum of eicosapentaeonic acid (EPA, 20:5 n-3), docosapentaeonic acid (DPA, 22:5 n-3) and docosahexaeonic acid (EPA, 22:6 n-3)
5. Sum of linoleic acid (LA, 18:2 n-6) and arachidonic acid (AA, 20:4 n-6)

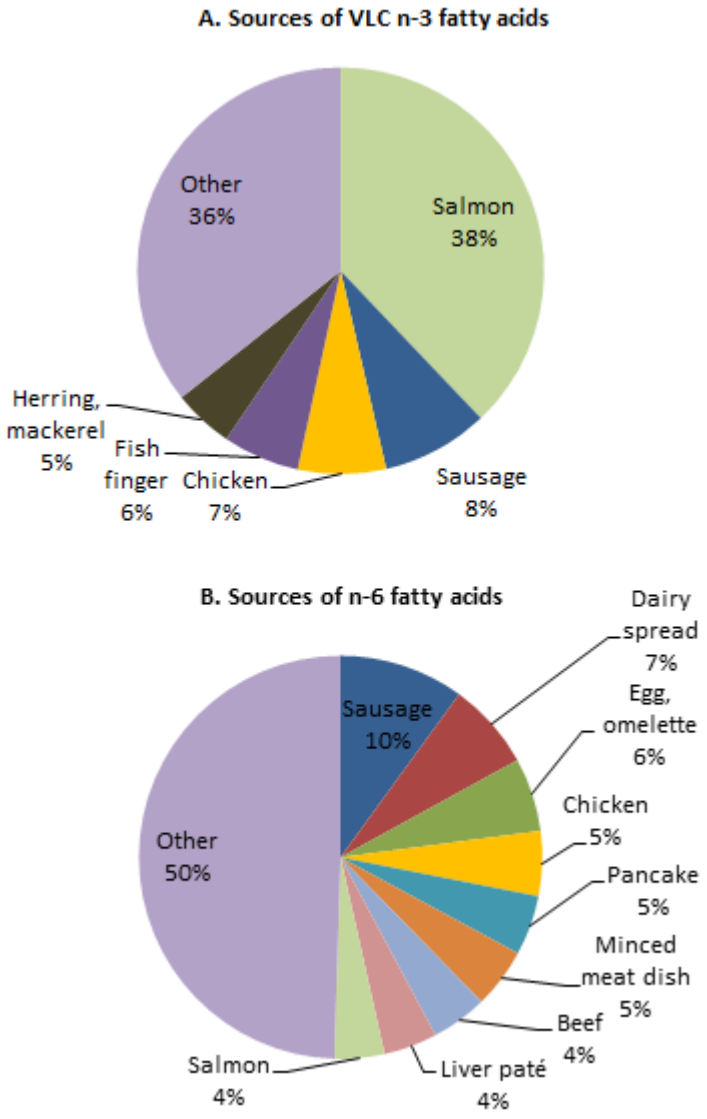

Figure 6.5 Sources for dietary consumption of total VLC n-3 fatty acids (A, sum of EPA, DPA and DHA) and n-6 fatty acids (B, sum of LA and AA) for the 1,970 children in the study population.

A higher intake of total VLC n-3 fatty acids was associated with a reduced risk of incident NAR between ages 8 and 16 years (OR_{adj}: 0.45 [95% CI: 0.30-0.67] for highest vs. lowest tertile, *P* trend<0.001). In addition, a higher ratio of n-6/n-3 fatty acids was associated with an increased risk of incident NAR (OR_{adj}: 1.56 [95% CI: 1.03-2.33] for highest vs. lowest tertile, *P* trend<0.032). No apparent associations were observed for total PUFA, total n-6 fatty acids and the single fatty acids ALA, LA and AA (see **Table IV** in **study III**). No evident associations were observed between vitamin D and incident AR or NAR.

Additional adjustment for early symptoms of allergic disease (wheeze and/or eczema during the first 2 years of life) did not affect the observed associations between total VLC n-3 fatty acids (OR_{adj}: 0.43 [95% CI: 0.28-0.65] for highest vs. lowest tertile), or the ratio of n-6/n-3 fatty acids (OR_{adj}: 1.56 [95% CI: 1.04-2.33] for highest vs. lowest tertile) and incident NAR.

In the same manner as for the analyses of fish, cases of AR and NAR were combined in post hoc analyses to raise the comparability with previously published results. A higher intake of total VLC n-3 fatty acids was associated with a reduced risk of incident rhinitis between ages 8 and 16 years (OR_{adj}: 0.73 [95% CI: 0.55-0.98] for highest vs. lowest tertile, *P* trend=0.034). In contrast, the association between the ratio of n-6/n-3 fatty acids and incident rhinitis was non-significant (OR_{adj}: 1.28 [95% CI: 0.96-1.74] for highest vs. lowest tertile, *P* trend<0.100).

Additional results not published in study III

Supplementation with VLC n-3 fatty acids was reported for 13 children at age 8 years and for 237 children at age 16 years. Additional adjustment for VLC n-3 fatty acid supplementation did not affect the association between intake of total VLC n-3 fatty acids at age 8 years and incident NAR between ages 8 and 16 years (data not shown).

In analyses of PUFA and vitamin D intake at age 8 years and AR and NAR at the same age, we observed that vitamin D intake was significantly associated with an increased risk of AR (**Table 6.2**). In addition, total PUFA and ALA were significantly associated with an increased risk of NAR.

Table 6.2 Odds ratios (ORs) of IgE-associated (AR) or non-IgE-associated (NAR) rhinitis at age 8 years[1] in relation to dietary PUFA (g/day) and vitamin D (µg/day) in tertiles at age 8 years (N=1,970)

	AR (n=263)	NAR (n=108)
	Adjusted OR[2] (95% CI)	**Adjusted OR[2] (95% CI)**
Total PUFA		
Q2	1.38 (0.96-1.98)	1.35 (0.78-2.34)
Q3	1.29 (0.83-1.98)	**2.16 (1.17-3.99)**
P for trend[3]	*0.344*	***0.012***
ALA		
Q2	1.10 (0.75-1.61)	1.69 (0.92-3.08)
Q3	1.29 (0.79-2.09)	**2.27 (1.07-4.83)**
P for trend[3]	*0.301*	***0.039***
VLC n-3		
Q2	1.01 (0.72-1.41)	1.09 (0.68-1.74)
Q3	1.20 (0.85-1.71)	0.70 (0.40-1.20)
P for trend[3]	*0.300*	*0.203*
n-6		
Q2	1.23 (0.83-1.82)	1.17 (0.64-2.14)
Q3	1.04 (0.63-1.71)	1.71 (0.84-3.47)
P for trend[3]	*0.961*	*0.106*
LA		
Q2	1.19 (0.80-1.77)	1.20 (0.65-2.20)
Q3	1.01 (0.61-1.68)	1.58 (0.77-3.26)
P for trend[3]	*0.897*	*0.188*
AA		
Q2	1.35 (0.95-1.92)	**1.71 (1.03-2.85)**
Q3	1.21 (0.81-1.81)	1.64 (0.90-2.99)
P for trend[3]	*0.439*	*0.123*
n-6/n-3 ratio		
Q2	0.89 (0.64-1.24)	0.94 (0.57-1.57)
Q3	0.90 (0.63-1.29)	1.34 (0.80-2.25)
P for trend[3]	*0.565*	*0.252*
Vitamin D		
Q2	**1.79 (1.11-2.89)**	1.92 (0.96-3.86)
Q3	**2.28 (1.03-5.01)**	1.94 (0.64-5.82)
P for trend[3]	***0.041***	*0.257*

P values of 0.05 or smaller are considered significant and shown in boldface

1. The reference group in analyses is children without sensitization and rhinitis symptoms at age 8 years
2. Model adjusted for intakes of carbohydrates, monounsaturated fatty acids, vitamin D, and the fatty acids used as exposures
3. Test for trend with median value of each exposure category

6.3.2 Plasma composition of polyunsaturated fatty acids and allergic disease at ages 8 and 16 years

The 940 children, included in **study IV**, were comparable with the children in the cohort, except that they had a somewhat higher prevalence of parental allergic disease (33.7% vs. 29.7%), and white collar workers among their parents (86.8% vs. 81.6%), and were fewer boys (45.7% vs. 50.5%) (see **Table E2** in the online repository of **study IV**). The inclusion criteria can be found in **Table 4.5**. The median proportion of total VLC n-3 fatty acids, ALA,

LA and AA, as percentage of total fat were 3.3 (IQR: 4.1-2.6), 0.2 (IQR: 0.28-0.19), 21.2 (IQR: 22.5-19.9) and 5.6 (IQR: 6.6-4.7), respectively, in plasma phospholipids at age 8 years. At age 16 years, 86, 413, and 419 children fulfilled the definition of asthma, rhinitis, and aeroallergen sensitization, respectively. The number of incident cases between ages 8 and 16 years of asthma, rhinitis, and aeroallergen sensitization were 43, 242, and 158, respectively.

In the analyses of plasma proportions of different PUFAs at age 8 years and allergic disease we used exposures as continuous variables. An increased proportion of total VLC n-3 fatty acids was inversely associated with asthma (OR_{adj}: 0.64 [95% CI: 0.50-0.82] per 1% increase), rhinitis (OR_{adj}: 0.73 [95% CI: 0.61-0.87] per 1% increase) and aeroallergen sensitization (OR_{adj}: 0.77 [95% CI: 0.66-0.90] per 1% increase) at the same age. Similar associations were observed for AA (OR_{adj}: 0.80 [95% CI: 0.67-0.96] per 1% increase, for asthma, OR_{adj}: 0.78 [95% CI: 0.68-0.88] per 1% increase, for rhinitis, and OR_{adj}: 0.76 [95% CI: 0.68-0.86] per 1% increase, for aeroallergen sensitization). No evident associations were observed for ALA and LA.

The associations between continuous plasma proportions of PUFAs at age 8 years and prevalent and incident allergic disease between ages 8 and 16 years can be seen in **Figure 6.6**. Increasing plasma proportion of total VLC n-3 fatty acids was inversely associated with asthma, rhinitis and aeroallergen sensitization at age 16 years, as well as with incident asthma between ages 8 and 16 years (OR_{adj}: 0.67 [95% CI: 0.47-0.94] per 1% increase).

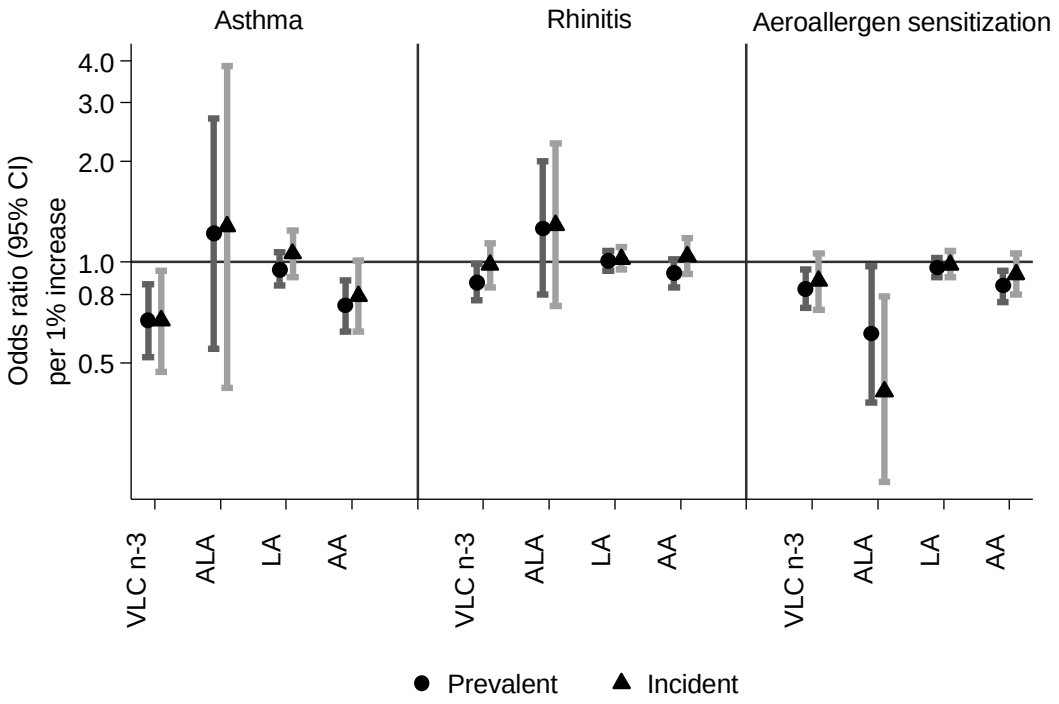

Figure 6.6 The associations between continuous plasma proportions of total VLC n-3 fatty acids, ALA, LA and AA and prevalent (circles) and incident (triangles) asthma, rhinitis, and aeroallergen sensitization between ages 8 and 16 years, in multivariable analyses adjusted for sex and parental allergic disease.

For ALA, we observed an inverse association with aeroallergen sensitization; the OR for incidence was 0.41 for an increase of 1% (95% CI: 0.22-0.79) (**Figure 6.6**). In contrast, LA was not associated with any of the outcomes. AA was inversely associated with asthma and aeroallergen sensitization, moreover, a borderline significant association with rhinitis was observed (OR_{adj}: 0.93 [95% CI: 0.84-1.02] per 1% increase). In addition, there was a borderline significant association between AA and a reduced risk of incident asthma (OR_{adj}: 0.79 [95% CI: 0.62-1.01] per 1% increase).

To investigate the possible impact of disease-related modification of exposure on the observed associations between plasma proportions of VLC n-3 fatty acids, ALA and AA and allergic disease at age 16 years, we additionally adjusted the analyses for early symptoms of allergic disease (wheeze and/or eczema during the first 2 years of life) and for symptoms of cow's milk allergy up to age 8 years. The adjustments had limited impact on the observed ORs (**Figure 6.7**).

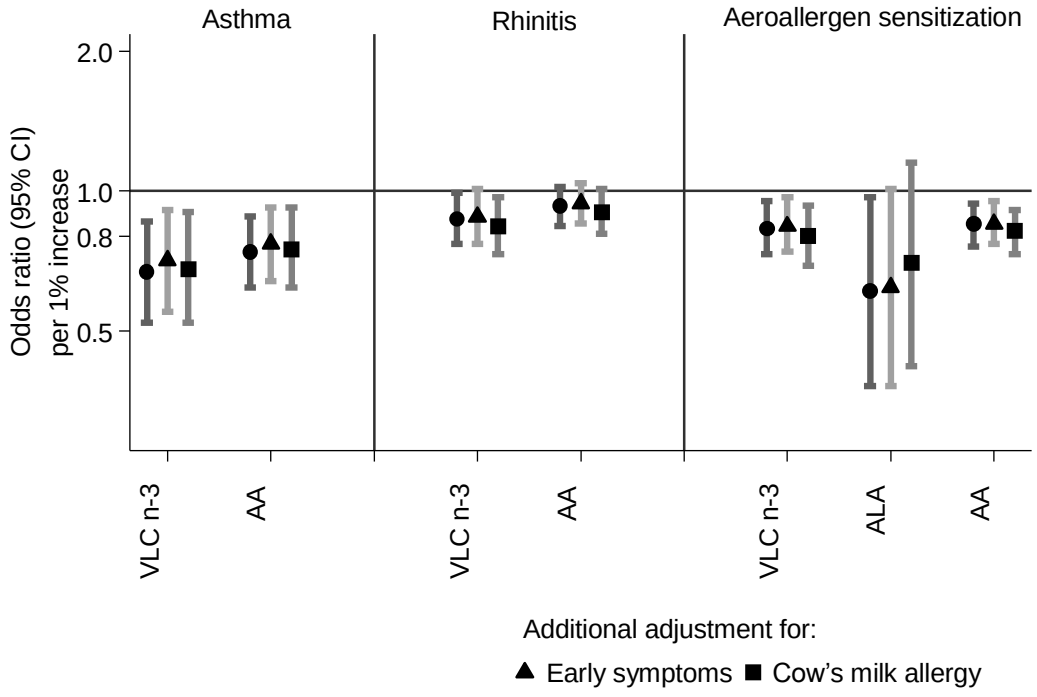

Figure 6.7 The associations between continuous plasma proportions of total VLC n-3 fatty acids, ALA, and AA and asthma, rhinitis and aeroallergen sensitization at age 16 years, in multivariable analyses adjusted for sex and parental allergic disease (circles). Additional adjustments were made for symptoms of allergic disease up to age 2 years (triangles) and for cow's milk allergy up to age 8 years (squares).

In analyses of phenotypes of allergic disease at age 16 years we observed an inverse association of total VLC n-3 fatty acids with IgE-associated asthma (OR_{adj}: 0.52 [95% CI: 0.38-0.71] per 1% increase), but not with non-IgE-associated asthma (OR_{adj}: 1.19 [95% CI: 0.75-1.88] per 1% increase) (**Figure 6.8**). Total VLC n-3 fatty acids was also significantly associated with IgE-associated rhinitis (OR_{adj}: 0.81 [95% CI: 0.69-0.94] per 1% increase),

while the association with non-IgE-associated rhinitis was non-significant (OR_{adj}: 0.92 [95% CI: 0.75-1.14] per 1% increase). For AA, we observed an inverse association with IgE-associated asthma (OR_{adj}: 0.61 [95% CI: 0.49-0.76] per 1% increase) and rhinitis (OR_{adj}: 0.85 [95% CI: 0.75-0.96] per 1% increase), but not with the non-IgE-associated phenotypes.

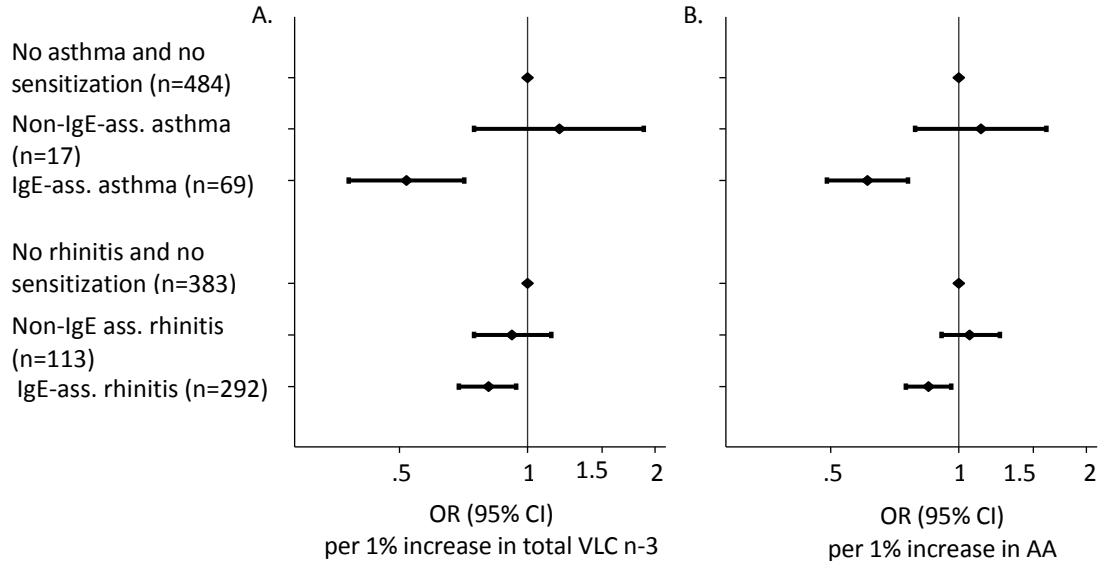

Figure 6.8 The associations between continuous plasma proportions of total VLC n-3 fatty acids (A), and AA (B) and non-IgE-associated, and IgE-associated phenotypes of asthma and rhinitis at age 16 years, in multivariable analyses adjusted for sex and parental allergic disease.

Additional results not published in study IV

Supplementation with VLC n-3 fatty acids was reported for 8 children at age 8 years and for 142 children at age 16 years. Additional adjustment for VLC n-3 fatty acid supplementation did not affect the observed associations between proportions of VLC n-3 fatty acids at age 8 years and asthma, rhinitis, and aeroallergen sensitization at age 16 years (data not shown).

6.4 THE RELATION BETWEEN CALCULATED INTAKE AND PLASMA PROPORTIONS OF POLYUNSATURATED FATTY ACIDS

The association between the calculated intake of the different PUFAs and the proportions measured in plasma phospholipids of the same PUFAs at the same age were estimated with the Spearman rank correlation test and the results are presented in **Table 6.3**. The correlations did not differ between children with or without allergic disease or aeroallergen sensitization at age 8 years.

Table 6.3 Spearman rank correlations between fatty acid intake (as percentage of total fat intake) calculated from a food frequency questionnaire and plasma proportions of fatty acids (as percentage of total amount fatty acids analyzed), stratified by allergic disease status at age 8 years[1]

	Study population (N=940)		Current allergic disease (n=343)		No current allergic disease (n=596)	
Fatty acid[2]	**r[3]**	***P*[4]**	**r[3]**	**95% CI**	**r[3]**	**95% CI**
ΣVLC n-3	0.23	<0.001	0.26	0.16-0.36	0.21	0.14-0.29
EPA	0.19	<0.001	0.23	0.13-0.33	0.17	0.09-0.24
DPA	0.04	0.28	0.06	-0.04-0.17	0.01	-0.07-0.09
DHA	0.29	<0.001	0.32	0.23-0.42	0.27	0.20-0.34
ALA	0.06	0.08	0.06	-0.05-0.16	0.06	-0.02-0.14
LA	0.02	0.46	-0.02	-0.12-0.09	0.05	-0.03-0.13
AA	0.08	0.01	0.10	-0.001-0.21	0.08	0.004-0.16

1. Asthma, rhinitis or aeroallergen sensitization

2. ΣVLC n-3: the sum of very long chain omega 3 fatty acids (Eicosapentaeonic acid [EPA, 20:5], Docosapentaeonic acid [DPA, 22:5] and Docosahexaeonic acid [DHA, 22:6]); ALA: α-linolenic acid, 18:3 n-3; LA: Linoleic acid, 18:2 n-6; AA: Arachidonic acid, 20:4 n-6

3. Spearman rank correlation

4. *P* value for the correlation

7 DISCUSSION

7.1 METHODOLOGICAL CONSIDERATIONS

7.1.1 Precision

The precision of a study can be explained as the degree to which the observed results are not due to chance. High precision means that repetition of the study would give the same results. The precision can be affected by random errors. Random errors originate from sampling variability and can be reduced by increasing the study size. The amount is reflected by the confidence intervals; wider confidence intervals imply more random error. The confidence intervals in **studies I-IV** are overall quite narrow. However, in some of the sub-analyses we observed very wide confidence intervals, which imply that we should be cautious in our interpretation of the results. This is the case for example in the stratification by parental allergic disease in **study I**.

7.1.2 Validity

A study with high validity measures what it is supposed to measure, the effect of systematic errors is small and the observed risk estimates are close to the true ones. Unfortunately, we will never know the true values; instead, we must try to reduce the systematic errors. Systematic errors that can be problematic in epidemiological studies are divided into three different categories: selection bias, information bias and confounding.

7.1.2.1 Selection

If the persons who choose not to participate in a prospective cohort have another frequency of exposure or outcome than the ones who choose to participate, their non-participation would result in a cohort not representative of the target population. Non-participation in cohort studies is unlikely to bias the associations between exposure and outcome since the outcome has not occurred at the time of inclusion and the associations are explored within the cohort. In any case, non-participation can affect generalizability and create a need to be careful about reporting prevalence rates, since they will not reflect the true prevalence rates in the general population.

The BAMSE birth cohort included 4,089 children out of 7,221 born in the catchment area during the recruitment period. A short questionnaire was sent out to 1,418 non-responders and excluded families to check possible selection bias, the response rate was 67%.[138] Parental smoking was more common among non-participants compared with participants, but no other characteristics differed, including parental allergic disease. Thus, the cohort is reasonably representative of the target population and the selection should not have led to bias in the associations under study.

Selection bias can, in longitudinal studies, be introduced at each follow-up. It is important to consider who has continued their participation, and who thereby has been eligible for the different studies. All longitudinal studies suffer from drop-out. Drop-out means that participants in the cohort decline to continue their participation and selection bias can be introduced if this depends on a specific factor. As seen in **Table 7.1** there were no major differences in the distribution of baseline characteristics between the original cohort and children followed up to ages 8 and 16 years, respectively. The participation rate has continued to be high in BAMSE all the way up to age 16 years, when 76% of the adolescents answered the questionnaire and 63% attended the clinical examination.

Table 7.1 Distribution of baseline characteristics among the children in the cohort, and the children followed up to ages 8 and 16 years[1]

	Cohort[2] (N=4,089)		Q8[3] (n=3,417)		CE8[4] (n=2,614)		Q16[5] (n=3,181)		CE16[6] (n=2,547)	
	n	%	n	%	n	%	n	%	n	%
Sex										
Boy	2,065	50.5	1,726	50.5	1,333	51.0	1,579	49.6	1,236	48.5
Maternal smoking										
Yes	563	13.8	440	12.9	334	12.8	415	13.1	310	12.2
Breastfeeding										
≥4 months	3,116	79.5	2,703	80.8	2,079	81.2	2,498	80.3	2,014	80.7
Socioeconomic status										
White collar worker	3,323	81.6	2,836	83.2	2,185	83.8	2,641	83.3	2,128	83.9
Parental allergic disease										
Yes	1,200	29.7	1,027	30.3	817	31.5	969	30.8	793	31.4
Eczema up to age 2y										
Yes	987	25.8	849	25.7	697	27.5	791	25.8	645	26.1
Any wheeze up to age 2y										
Yes	997	26.1	851	25.8	675	26.6	795	25.9	646	26.2

1. Some of the variables contain missing
2. Cohort refers to the original cohort of 4,089 children
3. Q8 refers to the group of children who responded to the 8-year questionnaire
4. CE8 refers to the group of children who attended the clinical examination at age 8 years and responded to the food frequency questionnaire
5. Q16 refers to the group of children who responded to the 16-year questionnaire
6. CE16 refers to the group of children who attended the clinical examination at age 16 years and left a blood sample

Selection bias can also be introduced by the inclusion criteria for the different studies included in this thesis. In comparisons of the prevalence of the different allergic diseases included in each study with the prevalence of that allergic disease in the cohort, I found them

to be similar overall (**Table 7.2**). However, the study population for **study IV** had slightly higher prevalence of all outcomes under study compared with the cohort, with the largest difference for specific aeroallergen sensitization at age 16 years (44.6% vs. 40.8%). On the other hand, only minor differences in the distribution of background characteristics have been observed between the specific study population and the cohort in both **study IV** and the other studies (see more details in the different studies, reproduced at the end of this thesis). The small differences are not likely to affect the association between exposure and outcome.

Table 7.2 Prevalence of allergic disease as defined in each study for the cohort and the different study populations (%)

Outcome	Cohort	Study I	Study II	Study III	Study IV
At age 8 years					
Asthma	6.3	6.0	6.4		9.4
Rhinitis[1]	13.6		13.7		
Rhinitis[2]	18.2				20.5
Eczema	12.9		12.9		
Any aeroallergen sensitization[3]	26.0	24.9	26.1		
Specific aeroallergen sensitization[4]	25.2				27.5
At age 12 years					
Asthma	6.6		6.8		
Rhinitis[1]	20.8		20.9		
Eczema	11.9		11.9		
At age 16 years					
Asthma	7.6				9.2
Rhinitis[2]	43.9				44.3
Specific aeroallergen sensitization[4]	40.8				44.6
Non-IgE-associated rhinitis	16.0			14.8	
IgE-associated rhinitis	21.2			21.2	

1. Prolonged rhinitis symptoms without a common cold in the past 12 months
2.*In addition to 1:* symptoms following contact with furred pets, pollens or mites
3. A positive Phadiatop, IgE ≥0.35kU/L
4. *In addition to 3:* at least one positive test to a specific aeroallergen (IgE≥0.35 k/UL)

7.1.2.2 Misclassification

Information bias rises from systematic errors in the measurement and classification of exposures and outcomes. If the misclassification of a variable is dependent on another variable the misclassification is said to be differential. A protective factor misclassified to be more common among the group with the outcome would lead to underestimation of the association. Moreover, a protective factor misclassified to be more common among the group without the outcome would lead to overestimation of the association. The same is true for differential misclassification of the outcome. Any misclassification not dependent on another factor is called non-differential and will only dilute the true association.

Misclassification of exposure

In the present studies, differential misclassification of exposure should be prevented by the longitudinal design; exposure data was collected before the outcome has occurred. Non-differential misclassification is possible, however.

Body mass index (BMI) was used as the exposure in **study I**. Weight and height measured by trained nurses during routine controls were registered at child and school care centers. The classification of normal vs. high BMI was based on the 85th percentile of BMI in the study population, taking age and gender into account, this step should not introduce any misclassification. Reference values to discriminate normal from high BMI are available; all take sex and age into account, but are based on different populations and cover different age spans. BMI discriminates subjects with a high weight for height, and it is debated how good of a measure BMI is at identifying adiposity. However, BMI has been shown to identify overweight in children to a high extent.[49, 50] In any case, BMI is not a good measure of fat mass distribution; to study that we need other measures.

Infant fish consumption was used as the exposure in **study II**. The frequency of fish consumption was asked for in the 1-year follow-up. Recent intake was reported, meaning that information bias should be a minor problem.

Fish consumption in childhood was used as the exposure in **study III**. All dietary measurement methods have inherent problems in capturing people's true intake. Here, I will discuss problems with food frequency questionnaires (FFQs) related to the measurement and classification of fish intake. Compared with persons with a regular fish intake, subjects who eat fish irregularly might find it harder to remember how often they have actually consumed fish in the past year and which types of fish were consumed, meaning that their exposure might be misclassified to a higher degree. Some types of fish are consumed seldom by most people and therefore easy to forget about when filling out a FFQ. However, the quantities can be large when these fish are consumed. An example is herring, which is usually eaten during our traditional festivals: Easter, Midsummer, and Christmas. Such irregular intake might not contribute much to the total amount of fish and might have a limited biological effect on the outcome. Eight-year-old children in Sweden are served school lunch and should be served fish at least once per week. Out of all children in the cohort, 83% report a fish intake of at least one time per week. Children might have trouble distinguishing between types of fish served in school. All these potential problems could have led to misclassification of fish intake. Nevertheless, any misclassification is most probable of the non-differential type, which leads to a dilution of a true association.

Consumption of polyunsaturated fatty acids (PUFAs) in childhood was used as exposure in **study III**. The estimated intakes of different PUFAs were calculated from the FFQ. In addition to the above discussion of problems connected to reporting of food intake in FFQs, additional potential problems concerns the nutrition calculation. Exact portion sizes of fish

intake were not asked about in the FFQ, instead portion sizes obtained from the Swedish national study Riksmaten 2003 were used. These might differ from the true portion sizes on an individual level but are hopefully representative on a group level, since Riksmaten 2003 includes the same age group that answering the FFQ in BAMSE. An additional potential problem can be to find nutrient composition values in the nutrient database which correspond to the reported food, and the nutrient values in the database might not be exact, since many factors affect nutrient content in for example fish (such as the way of farming and preparation methods). We compared the calculated intake levels of PUFAs with blood proportions of the same PUFAs, the correlation varied from 0.02 for linoleic acid (LA, 18:2 n-6) to 0.29 for docosahexaeonic acid (DHA, 22:6 n-3). The correlation for total very long-chain (VLC) n-3 fatty acids was 0.23, and 0.24 with reported oily fish intake. These moderate correlations can indicate poor assessment of PUFAs through the FFQ. On the other hand, blood proportions of PUFAs are affected by more than dietary intake and the measurement also has its limitations (see discussion below). The calculated consumption of PUFAs in BAMSE is slightly higher than was reported in Riksmaten 2003,[133] probably due to the different dietary assessment methods used. In **study III**, the calculated intake of different PUFAs was divided into tertiles. In that way, we could minimize the problem that we might not capture the absolute intake.

Plasma proportions of PUFAs were used as exposures in **study IV**. By using plasma to measure PUFAs, we could avoid the potential problems with dietary assessment methods. However, measurement in plasma can also be affected by a number of different factors. The blood sampling took place at the clinical examination at age 8 years; all those who attended were asked to provide a blood sample. Potential sampling variability should be random. Other factors that can possibly affect the plasma samples are storage, batch effects and laboratory measurement errors. These factors should affect samples from children with allergic disease the same as samples from children without allergic disease and therefore only introduce non-differential misclassification. Samples from children with allergic disease are more likely to have been thawed over the years compared with samples from children without allergic disease. Therefore, a higher degradation of PUFA can have taken place in samples from children with allergic disease. The observed inverse association between increasing PUFA proportions and a reduced risk of allergic disease might therefore be incorrect. In any case, the results of incidence of disease cannot be affected.

Disease-related misclassification of exposure

Even if **studies I-IV** are longitudinal and differential misclassification of exposure should be impossible, since the outcome under study had not happened when the exposure was assessed; disease-related modification of exposure could still be a problem. Parental allergic disease and early symptoms of allergic disease may affect which foods the children are served and may also affect overweight status; therefore, a possible impact of disease-related modification of exposure needs to be assessed in studies of allergic disease. This was done in all studies; by restriction or adjustment. In **studies I** and **II** we restricted analyses to children

without early symptoms of disease. This attenuated the observed ORs for the association between early high BMI and asthma at age 8 years in **study I,** and for the associations between early fish intake and asthma, and eczema in **study II**. Additional adjustment had minor impact on the observed associations in **studies I**, **III** and **IV**.

Misclassification of outcome

Some subjects will confuse colds with rhinitis symptoms, and wheeze can be due to airway infections. We used strict definitions to avoid misclassification. Information bias might be a problem; some perceive symptoms better while others do not remember them. Parents with allergic disease themselves might be more aware of symptoms in their children. Any misclassification is not likely to be related to the exposures under study in this thesis and is probably non-differential. In addition, we used immunoglobulin E (IgE) levels measured in blood samples as an objective marker to distinguish between IgE-associated and non-IgE-associated disease.

7.1.2.3 Confounding

Confounding is described in section 4.5.3. Uncontrolled confounding can lead to observation of associations that in reality are due to a confounding factor. Uncontrolled confounding can also mask true associations. Due to the extensive questionnaires we were able to test a range of potential confounders in each study. The factors were chosen based on a priori knowledge, and then their impact on the association between exposure and outcome was tested. Few factors changed the crude OR in my studies, the ones that did were adjusted for in a multivariable model. Despite the broad range of factors tested, residual confounding can still be present, due to either unmeasured confounding or measurement errors in measured confounders.

7.1.3 Generalizability

Based on the minor differences between the original cohort and the non-participants and between the different study populations and the original cohort (discussed in greater detail in section 7.1.2.1) the generalizability of the results to a Swedish population of children is good. The associations we have observed should be generalizable to most populations, given the belief that the underlying biological mechanisms are the same. Selection bias may have a greater influence if we want to compare prevalence rates. However, regarding **study I**, we should be cautious to generalize the observed results to an obese population (see section 4.2.3 for more information).

7.2 MAIN FINDINGS AND INTERPRETATIONS

7.2.1 Body mass index in relation to asthma and allergic sensitization at age 8 years

In **study I** we observed that children with a high BMI (≥the 85th percentile) during early childhood, but whose BMI normalized before age 7 years, had no increased risk of asthma. In contrast, children with a high BMI at age 7 years had an increased risk of asthma at age 8 years, regardless of their earlier BMI status. Moreover, a high BMI at age 7 years was associated with an increased risk of aeroallergen sensitization at age 8 years.

At the time of **study I** a number of longitudinal studies suggested high BMI to precede asthma in childhood.[73-78] However, to my knowledge, change in BMI status from early childhood had only been investigated in one previous study of 3,756 children from a Dutch birth cohort, in which they observed that early-transient high BMI was not associated with subsequent asthma, while a current high BMI was.[79] The results from **study I** were in line with the findings from the Dutch study. More recent longitudinal studies have shown contradictory results; some report that high BMI seems to precede asthma,[139] including a Mendelian randomization study,[140] while others have observed a bidirectional association.[141, 142]

A few studies have tried to disentangle if the timing of growth is of importance for asthma risk. A study using data from eight European birth cohorts observed that a rapid increase in BMI during the first 2 years of life was associated with an increased risk of incident asthma up to age 6 years.[143] There was also a tendency of an association for children with a persistent rapid growth up to age 6 years, however, there was less power to draw any conclusions from this group. In this same manner, a study of 9,723 children from an English birth cohort observed an increased risk of asthma up to age 17 years for children with a rapid weight growth during the first 3 months of age.[144] In contrast, a study based on a birth cohort from the United Kingdom, including 1,456 children, reported that early persistent and delayed high BMI was associated with asthma at age 18 years, while no association was observed for early-transient high BMI.[145]

Several meta-analyses of the association between overweight and asthma have been published. A recent review summarized meta-analyses and concluded that the current evidence suggest a mild-to-moderate causal effect of childhood overweight on asthma risk.[22] However, the included meta-analyses are published 2006 and 2013. Thus, the more recent studies are not included. Despite several longitudinal prospective studies which have investigated the association between overweight, mainly using BMI as a proxy, and asthma in childhood, there is still no clear picture of the temporal relation.[139-142] To come closer to the temporal relation it is important to disentangle the timing of onset of overweight contra onset of asthma; this is hard and needs to be further investigated.

There are few longitudinal studies of overweight and allergic sensitization. We revealed a significant association between a high BMI at school age and higher prevalence of aeroallergen sensitization at age 8 years in **study I**. This was not in line with two previous studies, which reported no association.[78, 79] In contrast, a recent study of 414 children who were followed for 2 years confirmed our findings.[146] Incident allergic sensitization was associated with an increase in BMI during the same period. Unfortunately, they did not distinguish between different types of sensitization.

A potential association between BMI and asthma and allergic sensitization is biologically plausible and most likely multifactorial. A number of review articles have discussed different possible mechanisms linking BMI with asthma in particular.[59, 147, 148] These include effects on lung function, upgraded inflammation through leptin, and behavioral factors. Despite several potential mechanisms suggested, the causal link is still unclear. Mutual risk factors have also been suggested, for example prenatal factors and genetic predisposition.[61, 149] The significant associations observed in **study I** between a high BMI and asthma and aeroallergen sensitization at school age, but not between early-transient high BMI and the outcomes, support the hypothesis that the two diseases share risk factors.

7.2.2 Fish consumption in relation to allergic disease

We observed that regular fish intake in infancy (≥2-3 times/month) reduced the risk of asthma, rhinitis and eczema up to age 12 years (**study II**). However, the inverse associations for asthma and eczema weakened after restriction to children without early symptoms of allergic disease, but the association for rhinitis was not affected. In addition, regular oily fish consumption at age 8 years (≥1 time/week) was associated with a reduced risk of developing rhinitis between ages 8 and 16 years, also after adjustment for early fish intake and early symptoms of allergic disease (**study III**).

At the time of **study II**, several longitudinal studies had investigated the role of fish intake in infancy in relation to allergic disease up to age 4 years. The majority observed an inverse association between early introduction or frequent intake of fish in infancy and subsequent allergic disease (asthma, rhinitis, eczema, or allergic sensitization) during preschool age,[95, 97-102] including a previous report from BAMSE.[96] In this same manner, two other longitudinal studies reported an increased risk of wheeze at age 3 years and aeroallergen sensitization at age 5 years among children with delayed introduction of fish.[150, 151] One prospective study based on a Dutch birth cohort reported no association between fish consumption at age 2 years and asthma or wheeze at age 3 years.[152] To my knowledge, two other studies have investigated the association of infant fish intake with allergic disease beyond pre-school age.[118, 153] In one of the studies, based on 4,051 children from a Swedish birth cohort, introduction of fish before 9 months of age was observed to reduce the risk of prevalent asthma at age 8 years.[153] In contrast, the other study including 3,269 children from a Dutch birth cohort, reported no association between fish intake at age 2 years and prevalent asthma

at age 8 years.[118] The discrepancies can be due to the amount of different fish species consumed, the definition of outcome, or the timing of exposure and outcome. Most of these studies took parental allergic disease and early symptoms of allergic disease into account by adjustment or restriction, which is of importance since disease-related modification of exposure can affect introduction and consumption of fish in early life. Parents with allergic disease, or who see signs of early symptoms in the children, might delay the introduction of fish to their infant's diet. When the children in BAMSE were newborns the recommendation was to delay introduction of fish for infants at increased risk of allergic disease.[37] We could see that parental allergic disease and early symptoms of allergic disease delayed the introduction and reduced the amount of fish in the child's diet at age 1 year, which has also been reported previously in a study from BAMSE.[96] We handled this by adjusting all analyses for parental allergic disease and in a second step we restricted the analyses to children without early symptoms of allergic disease. The inverse associations for asthma, and eczema weakened after restriction, but the association for rhinitis was not affected. The recommendation to delay the introduction of allergenic foods has since been removed.[38]

Fish consumption in infancy seems to be a window of opportunity to influence the risk of allergic disease, maybe through the content of immuno-modulatory factors in oily fish, for example vitamin D or VLC n-3 fatty acids (possible mechanisms are discussed below). At the same time, the impact of fish intake in infancy is difficult to disentangle from a possible influence of maternal intake during pregnancy on the risk of childhood allergic disease.[33, 154] We could unfortunately not adjust for maternal fish intake during pregnancy in **study II** due to lack of information. Therefore, our finding of an association between regular infant fish intake and subsequent allergic disease might be due to a high exposure of fish in utero. Studies that could take consumption during pregnancy into account have observed independent associations for infant intake.[100, 151]

Fish consumption later in childhood can also play a role for the risk of childhood allergic disease, since the occurrence and incidence is high up into adolescence.[3, 155] Intake of fish at school age has been associated with a reduced risk of asthma and allergic sensitization in cross-sectional studies,[103-112] with some exceptions.[113-117] However, inverse associations have not been observed for rhinitis.[104, 107, 113, 115, 116] We did not observe an association between fish intake at age 8 years and rhinitis at the same age in **study III**. In longitudinal analyses, we found a reduced risk of incident rhinitis between ages 8 and 16 years for children with regular intake of oily fish. **Study III** was the first longitudinal study that investigated the association between fish consumption and risk of developing rhinitis all the way up to adolescence; our findings need to be confirmed or refuted in future longitudinal studies. The result from **study III** was not affected by infant fish intake and could not be explained by disease-related modification of exposure.

One explanation for the observed association between intake of oily fish and allergic disease could be that fish is part of a healthy lifestyle in general, which in itself lowers the risk of

allergic disease. We tried to adjust our analyses for a number of lifestyle factors, for example other dietary factors, like fruit, vegetable, and meat consumption. None of the other dietary factors could explain the inverse association between oily fish and development of rhinitis.

The mean intake of fish in the study population was in line with the intake reported in the Swedish national report Riksmaten 2003 for the same age group (1.70 vs. 1.75 times per week).[133] An intake of oily fish once per week or more, which was associated with a reduced risk of allergic disease, is in line with the current dietary guidelines to consume different types of fish at least twice a week.[85]

An inverse association between oily fish and allergic disease, including rhinitis, is biologically possible and might be explained by the rich amount of vitamin D in oily fish. Vitamin D has immuno-modulatory properties.[90] We did not observe an association between intake of vitamin D at age 8 years and incident rhinitis between ages 8 and 16 years in **study III**. Vitamin D intake is low in general, including among our study participants, and the status of vitamin D is therefore highly determined by sun exposure.[90] Blood levels of vitamin D are widely used instead of intake data, but unfortunately these levels have not been measured in BAMSE. A recent review of observational studies concluded that the evidence for an association between serum vitamin D and allergic disease in childhood is lacking, but added that vitamin D seems to reduce the severity of asthma.[156]

Other constituents of oily fish which might explain the reduced risk of allergic disease are the VLC n-3 fatty acids EPA, DPA, and DHA. These are discussed in the next section. Regardless of what components in fish that exert the influence on allergic disease we observed infant fish consumption to reduce the risk of rhinitis, in particular, up to age 12 years (**study II**) and fish intake at school age to reduce the risk of incident rhinitis between ages 8 and 16 years (**study III**).

7.2.3 Polyunsaturated fatty acids in relation to allergic disease

In **study III** we reported that a higher intake of total VLC n-3 fatty acids at age 8 years was associated with a reduced risk of developing rhinitis between ages 8 and 16 years, in particular non-IgE-associated rhinitis. Furthermore, we found inverse associations between increased plasma proportions of total VLC n-3 fatty acids at age 8 years and asthma, rhinitis and aeroallergen sensitization at ages 8 and 16 years in **study IV**. Increased plasma proportion of total VLC n-3 fatty acids was also associated with a reduced risk of incident asthma between ages 8 and 16 years. Increasing plasma proportions of AA was inversely associated with asthma and aeroallergen sensitization at ages 8 and 16 years. The inverse associations between plasma proportions of total VLC n-3 fatty acids and AA and allergic disease were most pronounced for the IgE-associated phenotypes.

Previous studies of dietary PUFA and allergic disease in childhood have been cross-sectional. Studies of intake of total PUFA have reported no association for rhinitis,[129, 157] but have observed an increased risk of wheeze or asthma among children with higher intake of PUFA.[127, 128, 157, 158] The results from analyses of consumption of specific n-3 and n-6 fatty acids have been inconclusive. No significant associations have been reported between VLC n-3 fatty acid intake and asthma or rhinitis.[117, 127-129] A Japanese survey, based on around 25,000 children between ages 6 and 15 years, observed an association between higher intake of LA and increased risk of wheeze,[127] and an inverse association between increasing AA intake and rhinitis at school-age.[129] For comparison, I performed cross-sectional analyses for **study III** and observed significant associations between the highest tertile of total PUFA and ALA consumption and an increased risk to have non-IgE-associated rhinitis at age 8 years.

In contrast to dietary PUFAs, increased proportions of total VLC n-3 fatty acids in plasma at age 8 years was inversely associated with rhinitis, asthma, and aeroallergen sensitization at the same age in **study IV**. This finding was in line with previous cross-sectional studies of blood composition, which reported lower blood proportions of VLC n-3 fatty acids in allergic subjects than in controls.[104, 130, 131] However, some did not observe any differences for VLC n-3 fatty acids.[112, 159] In one recent international study of 2,400 children, associations between total n-6 fatty acids, and DPA and increased risk of current allergy in children aged 2-9.9 years were observed.[160] In age stratified analyses the associations were confined to the youngest age group (2-3.9 years). Our finding of inverse associations between AA and allergic disease at age 8 years is not supported by previous studies; no differences in LA and AA proportions between allergic cases and controls have been observed.[104, 112, 131, 159, 161] One Korean study observed increased levels of AA in allergic children.[130]

Increased plasma proportions of total VLC n-3 fatty acids at age 8 years was observed to reduce the risk of asthma, rhinitis, and allergic sensitization at age 16 years, as well as the risk of incident asthma between ages 8 and 16 years, in **study IV**. A recent study from Finland reported a similar inverse association between higher proportions of EPA, DHA and total VLC n-3 fatty acids in serum during preschool age and a reduced risk of developing asthma up to age 5 years.[162] However, additional adjustment for cow's milk allergy attenuated the findings and only the association for EPA remained significant. Disease-related modification of exposure did not have the same influence on the associations in our study; adjustment for symptoms of cow's milk allergy up to age 8 years and adjustment for early symptoms of allergic disease did not affect the observed associations in **study IV**. We observed the most pronounced findings for the IgE-associated phenotypes of allergic disease in **study IV**. In contrast, the significant association for EPA was most apparent for the non-IgE-associated phenotype of asthma in the Finish study. An explanation for the differences can be the older age of the children in our study. Disease-related modification of exposure might have a smaller influence in older children and allergic sensitization increase with increasing age.[163] The inverse association between a higher proportion of AA and allergic disease observed in

study IV was not found in the Finnish study. To my knowledge, there are no other published studies on blood proportions at school-age and development of allergic disease.

An inverse association between VLC n-3 fatty acids and allergic disease is biologically plausible. VLC n-3 fatty acids have been demonstrated to have a range of anti-inflammatory effects; they seem to lower the production of inflammatory mediators and increase the level of anti-inflammatory mediators.[123] The n-6 fatty acid AA has been shown to have both pro- and anti-inflammatory effects, for example through the prostaglandins (PGs) D_2 and E_2.[7] Both PGD_2 and PGE_2 have been labeled as pro-inflammatory, but in the respiratory system PGE_2 seems to have beneficial effects, such as protection against bronchoconstriction and inhibited recruitment of inflammatory cells. Therefore, our finding of an inverse association between higher proportions of AA in plasma phospholipids and allergic disease is plausible. We could not observe the same finding for dietary intake of AA, but this might be due to the low consumption of AA in our study population.

In summary, both the calculated dietary intake and plasma proportions of total VLC n-3 fatty acids were associated with a reduced risk of subsequent allergic disease (**studies III** and **IV**). In addition, we observed plasma proportions of AA to be associated with a reduced risk of allergic disease in **study IV**.

7.2.4 The relation between calculated intake and plasma proportions of polyunsaturated fatty acids

The correlations between the different PUFAs, as calculated intakes from the FFQ filled out at age 8 years and as proportions measured in plasma phospholipids from the same age, were moderate. LA, ALA and VLC n-3 fatty acids are considered to be good biomarkers of dietary intake, since LA and ALA are essential fatty acids not endogenously produced in the human body and the conversion rate of VLC n-3 fatty acids is low in most humans.[121] One explanation for the moderate correlations observed in **study IV** may be that the long-term storage of the plasma samples has led to degradation of the fatty acids. However, the samples were stored in minus 80ºC, a temperature at which the fatty acid composition should be stable over time.[136] Another possible explanation is that the FFQ and plasma phospholipid fraction correspond to slightly different time spans. In the FFQ the intakes over the previous 12 months were asked for, while plasma phospholipids reflect more recent intake.[136] In addition, fatty acids are affected by absorption and metabolism and therefore classified as concentration biomarkers, meaning that correlation coefficients between them and estimated dietary intake will be substantially lower than one, regardless of perfect assessment of dietary intake.[164] Validation studies in adults have reported correlation coefficients between 0.20-0.60 for the different VLC n-3 fatty acids calculated from FFQ and measured in blood.[165] The correlation coefficients reported from validation studies in children have been in the lower range (0.20-0.30).[166-168]

8 CONCLUSIONS

In the studies included in this thesis we found that:

- High body mass index during the first 4 years of life was not associated with risk of asthma during school age among children who had developed a normal weight by 7 years of age. (**Study I**)

- High body mass index at age 7 years was associated with an increased risk of asthma and aeroallergen sensitization at school age. (**Study I**)

- Fish intake may reduce the risk of subsequent allergic disease. (**Studies II** and **III**)
  More specifically:
  A regular fish consumption in infancy seemed to reduce the risk of rhinitis during childhood, also after adjustment for fish intake at school age. Early symptoms of allergic disease attenuated the inverse associations for asthma and eczema. (**Study II**)
  A regular intake of oily fish at school age seemed to reduce the risk of developing rhinitis in adolescence. (**Study III**)

- Polyunsaturated fatty acids in childhood appeared to influence the risk of allergic disease in adolescence. (**Studies III** and **IV**)
  More specifically:
  A high proportion of VLC n-3 fatty acids, in diet and plasma, seemed to reduce the risk of subsequent allergic disease. (**Studies III** and **IV**)
  A high proportion of AA in plasma seemed to reduce the risk of subsequent allergic disease. (**Study IV**)

Overall, these studies indicate that body mass index is associated with concurrent asthma and aeroallergen sensitization at school age. Moreover, consumption of fish, both in infancy and childhood, and polyunsaturated fatty acids, especially VLC n-3 fatty acids, appear to be associated with a reduced risk of subsequent allergic disease throughout childhood.

9 FUTURE PERSPECTIVES

Results from the studies included in this thesis have added to previous knowledge from epidemiological research. In epidemiology, a single study may indicate an association, but the accumulated knowledge from multiple studies can help us draw conclusions about associations between exposures and outcomes.

A great number of studies have tried to disentangle the association between overweight and asthma during childhood. However, few studies assessed both body mass index (BMI) and asthma at several time points from infancy to adolescence which is needed to come closer to the temporal relationship. To be able to investigate all the different trajectories of overweight and asthma with enough power, international collaborations are probably needed.

Most of the previous studies have like us used BMI as a proxy of overweight. To further understand the mechanisms behind an association between overweight and asthma, longitudinal studies with more precise measures of adiposity distribution and fat mass are needed. Moreover, the association between overweight and allergic sensitization need to be further investigated. Our finding of an association between a high BMI and aeroallergen sensitization at school age need to be replicated or refuted, preferably in studies of incident sensitization.

Despite an increasing number of studies on timing of introduction of food and allergic disease, there are few longitudinal studies of fish or polyunsaturated fatty acid (PUFA) intake in childhood and subsequent risk of allergic disease up to adolescence. Therefore, our observed associations between consumption of oily fish and PUFAs in childhood and a reduced risk of incident allergic disease up to age 16 years need to be replicated or refuted in other cohort studies. Future studies of fish and PUFAs should strive to separate the analyses for the different types of fish and PUFAs, as we have done, since the associations and biological mechanisms probably differ. To be able to study specific intake, diet needs to be assessed through a detailed questionnaire, and to further enhance the exposure assessment, a combination with biomarkers of intake would be preferred.

Fish and PUFAs are not consumed in isolation, but make up part of an overall diet. Synergistic effects and diet-gene interactions possibly occur and need to be considered in future research. Another aspect of diet is food choice. Subjects with high intake of fish, for example, must consume less of something else. It would be interesting to investigate if the strength of the association with allergic disease differs depending on what food items subjects with high fish intake consume less of. And in addition, to investigate if the association between a high fish intake and a reduced risk of allergic disease differs depending on what the subjects in the reference group with a low fish intake consume instead.

Finally, I hope that the findings in this thesis will contribute to the knowledge about modifiable factors for the risk of allergic disease in children, and inspire new research within the area that in the end can lead to suggestions to families and society on how to prevent allergic disease.

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