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Diet and dietary biomarkers during pregnancy and lactation in relation to offspring allergy development

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ABSTRACT

Allergy causes a large burden on society and reduces the quality of life for the individual. The increased allergy incidence over the last decades cannot be explained by genetics. Research has indicated that diet, which is a modifiable factor, might influence the risk of developing allergy. Traditional dietary assessment methods are, however, prone to large measurement errors. Objective biomarkers may be complementary but have typically not been applied in cohorts with pregnant women and in the early life context.

The objective of this thesis was to investigate if the diet during pregnancy and lactation is related to offspring allergy development (i.e., atopic eczema, food allergy, and asthma) diagnosed by an allergologist at twelve months of age. The thesis is based on data from the birth cohort Nutritional impact on Immunological maturation during Childhood in relation to the Environment (NICE). Dietary data were collected using a repeated web-based semi-quantitative food frequency questionnaire sent out in gestational week 34, one month postpartum, and four months postpartum. Dietary intakes were quantified based on pictures of portion sizes and reported intake frequency. Maternal and infant blood, urine, and breast milk samples were collected, and nutrients, trace elements, and metabolomics-based food intake biomarkers were related to the self-reported food intake and to allergy diagnosis.

The results show that maternal intake of cow's milk products and saturated fat during lactation was associated with a lower incidence of offspring allergy. Higher proportions of n-6 polyunsaturated fatty acids in umbilical cord plasma phospholipids correlated to a higher incidence of atopic eczema during the first year of life. In addition, food intake during pregnancy was associated with maternal characteristics, primarily age and educational level. Food intake biomarkers known from a general (i.e., non-pregnant or lactating) population seemed useful also during lactation, whilst dietary biomarkers during pregnancy warrant further investigation.

In summary, the findings indicate that the most crucial period of time in terms of allergy prevention may be the first months postpartum, rather than during pregnancy. Hence, changing maternal diet during lactation may be a useful strategy for allergy prevention in the offspring.

Keywords: Pregnancy, Breastfeeding, Infancy, Diet, Cohort, Dietary biomarkers, Metabolomics, Allergy, Food allergy, Atopic eczema.

LIST OF PUBLICATIONS

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

I. **Stråvik M**, Jonsson K, Hartvigsson O, Sandin A, Wold AE, Sandberg A-S, Barman M. Food and Nutrient Intake during Pregnancy in Relation to Maternal Characteristics: Results from the NICE Birth Cohort in Northern Sweden. Nutrients. 2019, 11(7).

II. **Stråvik M**, Barman M, Hesselmar B, Sandin A, Wold AE, Sandberg A-S. Maternal intake of cow's milk during lactation is associated with lower prevalence of food allergy in the offspring. Nutrients. 2020, 12(12).

III. Barman M, **Stråvik M**, Broberg K, Sandin A, Wold AE, Sandberg A-S. Proportions of polyunsaturated fatty acids in umbilical cord blood at birth are related to atopic eczema development in the first year of life. Nutrients. 2021, 13(11).

IV. **Stråvik M**, Gustin K, Barman M, Levi M, Sandin A, Wold AE, Sandberg A-S, Kippler M, Vahter M. Biomarkers of seafood intake during pregnancy – pollutants versus micronutrients and fatty acids. Environmental Research. 2023, 225(2023).

V. **Stråvik M**, Hartvigsson O, Sandin A, Wold AE, Barman M, Sandberg A-S. LC-MS based metabolomics for dietary biomarker discovery in a cohort of pregnant and lactating women and their infants. *Manuscript in preparation*.

Published papers not included in the thesis:

- **Stråvik M**, Gustin K, Barman M, Skröder H, Sandin A, Wold AE, Sandberg A-S, Kippler M, Vahter M. Infant Iodine and Selenium Status in Relation to Maternal Status and Diet During Pregnancy and Lactation. Frontiers in Nutrition. 2021; 8:733602.

- Gustin K, Barman M, **Stråvik M**, Levi M, Englund-Ögge L, Murray F, Jacobsson B, Sandberg A-S, Sandin A, Wold AE, Vahter M, Kippler M. Low-level maternal exposure to cadmium, lead, and mercury and birth outcomes in a Swedish prospective birth-cohort. Environmental Pollution. 2020:114986.

- Kampouri M, Gustin K, **Stråvik M**, Barman M, Levi M, Daraki V, Jacobsson B, Sandin A, Sandberg A-S, Wold AE, Vahter M, Kippler M. Association of maternal urinary fluoride concentrations during pregnancy with size at birth and the potential mediation effect by maternal thyroid hormones: The Swedish NICE birth cohort. Environmental Research. 2022:114129.

CONTRIBUTION REPORT

Paper I: Mia Stråvik (MS) collected the dietary data, curated the raw data, calculated estimated portion sizes for quantification of dietary intake, wrote the original draft of the manuscript, performed data analysis, and revised the manuscript together with the coauthors.

Paper II: MS collected the dietary data, collected additional information on timepoint of early allergy symptoms, curated the raw data, quantified the dietary intake, wrote the original draft of the manuscript, performed data analysis and visualization, and revised the manuscript together with the coauthors.

Paper III: MS collected, curated, and quantified the dietary data, performed data analysis and visualization, and revised the manuscript together with the coauthors.

Paper IV: MS collected, curated, and quantified the dietary intake, wrote the original draft of the manuscript, performed data analysis and visualization, and revised the manuscript together with the coauthors.

Paper V: MS collected, curated, and quantified the dietary data, worked with identification of the metabolites, wrote the original draft of the manuscript, performed data analysis and visualization, and revised the manuscript together with the coauthors.

ABBREVIATIONS

CI	Confidence interval
CMPF	3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid
FFQ	Food frequency questionnaire
FoodBAll	Food Biomarker Alliance
LCPUFA	Long-chain polyunsaturated fatty acid
Meal-Q	A web based semiquantitative food frequency questionnaire
MS	Mass spectrometry
n-3	Omega-3
n-6	Omega-6
NICE	Nutritional impact on Immunological maturation during Childhood in relation to the Environment
OR	Odds ratio
SPT	Skin prick test

TABLE OF CONTENTS

1. INTRODUCTION

The purpose of epidemiologic research is to assess disease incidence and to identify factors contributing to that incidence. Allergy affects millions of people worldwide, and it is well-documented what negative impact it can have on the quality of life and societal economy [1]. Genes, diet, and microbial exposure have an impact on whether allergy develops or not, but exactly how this works is unknown. Importantly, diet is a factor that can be modulated. The role of diet is difficult to measure and is often questioned due to the challenges of measuring food intake accurately. Traditional dietary assessment methods (i.e., based on interviews and questionnaires) are prone to measurement errors and are subjective since they e.g., rely on human memory. One way to minimize these drawbacks and get closer to assessing the actual food intake is to measure the intake in combination with more objective methods where biomarkers are quantified in biological samples.

Although allergies affect a significant number of families, established strategies for preventing these diseases are still unavailable. The understanding of who will develop allergy, and who will not, is lacking although allergic heredity can hint about which child will be at higher risk. Three decades ago, in the year of 1989, the hygiene hypothesis was formulated [2]. The theory suggested, based on observations, that childhood infections might protect against allergies. Genetics, diet, and exposure to microorganisms shape the immune system during the perinatal period, which can be seen as a "window of opportunity" for immune maturation [3]. In this window, while the immune system is still developing, environmental factors (including diet) might be crucial [4, 5]. A few years after the hygiene hypothesis was launched, in the year of 1993, a theory regarding a change in dietary fatty acids was formulated when increased observations of asthma were seen together with a shift in dietary habits from omega-3 (n-3) to omega-6 (n-6) dominated food [6, 7]. Regarding food allergy in particular, a hypothesis referred to as the dual-allergen exposure hypothesis suggests that tolerance towards a potential food allergen can be achieved if the first exposure occurs via the mouth and not via a damaged skin barrier [8]. Hence, a combination of home environment and diet during both pregnancy and lactation could explain part of the allergy risk. In this thesis, the dietary part will be in focus.

Since allergies manifest differently over time, it is important to do epidemiologic research over several phases of life. Further, food intake must be closely followed and thoroughly assessed to draw any conclusions regarding its role in disease development. Studies investigating these associations rarely assess diet repeatedly from pregnancy and onwards. In addition, studies on food intake biomarkers have not focused on pregnant and breastfeeding women which have left a gap in knowledge regarding more objective measurements of diet at this period in life.

2. OBJECTIVES

The primary aim was to investigate if dietary intake during pregnancy and lactation is associated with offspring allergy development (i.e., atopic eczema, food allergy, and asthma) during the first year of life in the Swedish birth cohort Nutritional impact on Immunological maturation during Childhood in relation to the Environment (NICE), using a combination of subjective and objective dietary assessment methods (**Figure 1**). More specifically:

a) Investigate maternal characteristics and lifestyles associated with dietary intake during pregnancy (**Paper I**)
b) Investigate if dietary intake during pregnancy and lactation is associated with offspring allergy (**Paper II** and **Paper III**)
c) Explore candidate food intake biomarkers during pregnancy and lactation in relation to diet and offspring allergy, applying targeted biomarker analyses (**Paper II, Paper III**, **Paper IV)** and metabolomics (**Paper V**)

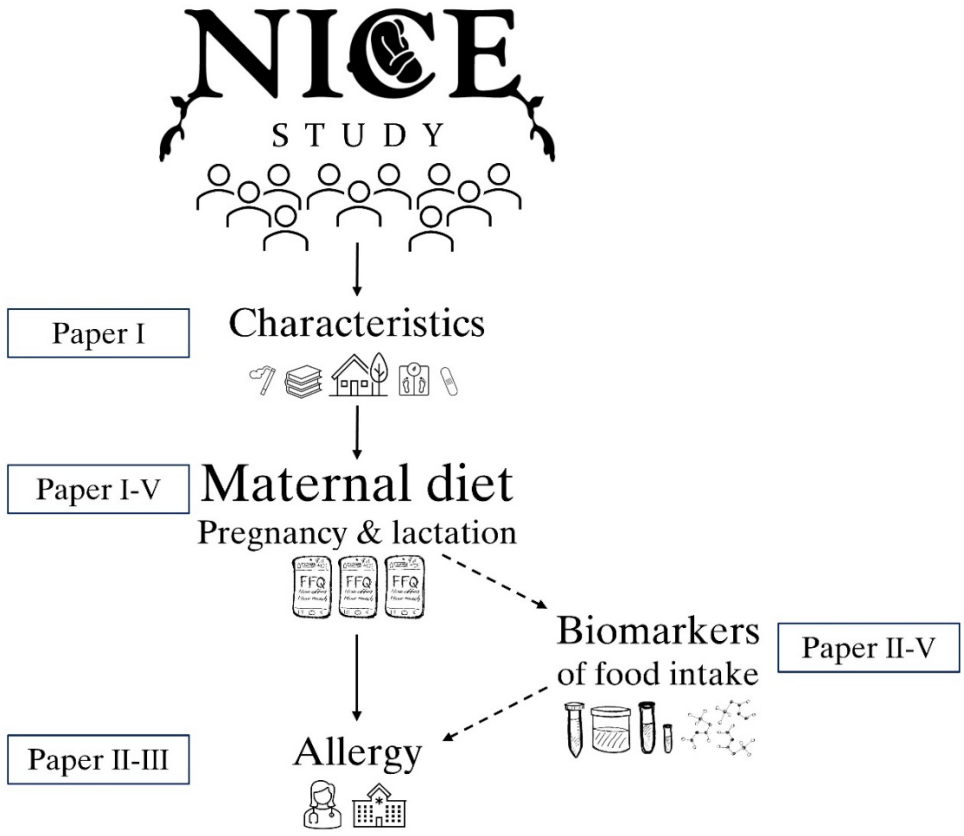

Figure 1. Overview of the papers included in the thesis.
Family characteristics (e.g., sociodemographic factors, home environment, allergies) were collected around gestational week 18. Maternal diet was assessed using repeated semi-quantitative food frequency questionnaires reflecting intake in gestational weeks 30-34, first and fourth months postpartum. Biomarkers of food intake were measured in erythrocytes, plasma, urine, and breast milk. Allergy was diagnosed at 12 months of age by the study pediatrician specialized in allergy.

3. BACKGROUND

Dietary habits have been associated with allergy development in several studies [9-11]. However, such associations are often questioned due to the difficulties in measuring food intake accurately. The fact that it is difficult to assess diet is one of the main challenges when it comes to estimating the role of diet in disease prevention [12]. When it comes to allergy as an outcome in these types of studies, the lack of allergologist diagnosis could affect these associations even further.

3.1. Allergic diseases

Allergy is a broad concept including several diseases such as food allergy, atopic eczema, asthma, and hay fever [13]. Although the term includes several different conditions, they are closely related. The atopic march explains how different allergic conditions often develop over time. The atopic march states that allergy manifests in different ways throughout life, starting with atopic eczema and certain types of food allergies (e.g., cow's milk and egg) during infancy, to progress into allergic rhinitis and asthma later in life [14].

The etiology of allergic diseases is not yet fully understood. It appears to be a combination of genetic and environmental factors. Allergy can be described as a hypersensitivity reaction to an exogenous substance, an allergen, that normally would not cause the immune system to react [13]. An allergen is an antigen that causes an allergic reaction in the body. Allergens are often proteins and can be airborne (e.g., pollen, animal dander, dust mite), or, for instance, be found in foods (e.g., cow's milk, egg, and peanut) [15]. However, some allergic reactions are triggered by a carbohydrate (galactose-α-1,3-galactose in red meat) [16].

At first exposure to an antigen (e.g., an allergen), it is presented to T cells and B cells in the lymph nodes. These immune cells then mature into different subsets as a response to the antigen exposure. Depending on how the T cell matures, different cytokines are produced and released, which further stimulate (cytokines produced by T helper (Th) 2 cells) or inhibit (cytokines produced by the Th1 cells) the B cells to differentiate into antibody-producing plasma cells. In the allergic sensitization phase, allergen-specific immunoglobulin (Ig) E antibodies are created by plasma cells. These IgE antibodies bind to receptors on the surface of mast cells. This first part is referred to as sensitization (**Figure 2**). Sensitization *per se* is not necessarily a problem since one can be sensitized without experiencing any allergic symptoms. In the NICE cohort, for instance, 64% (N=23) of the sensitized infants developed any type of allergy (i.e., food allergy, eczema, asthma, rhinitis, and conjunctivitis).

At second exposure to the allergen, the immune system is ready to react. The allergen now binds directly to the IgE antibodies attached to the mast cells. This causes the release of inflammatory substances (primarily histamine) from the mast cells (**Figure 2**). The release of histamine is what causes the typical allergic symptoms (i.e., runny nose, itchy eyes, swollen lips). The allergic symptoms depend on where the histamine is released and to what extent.

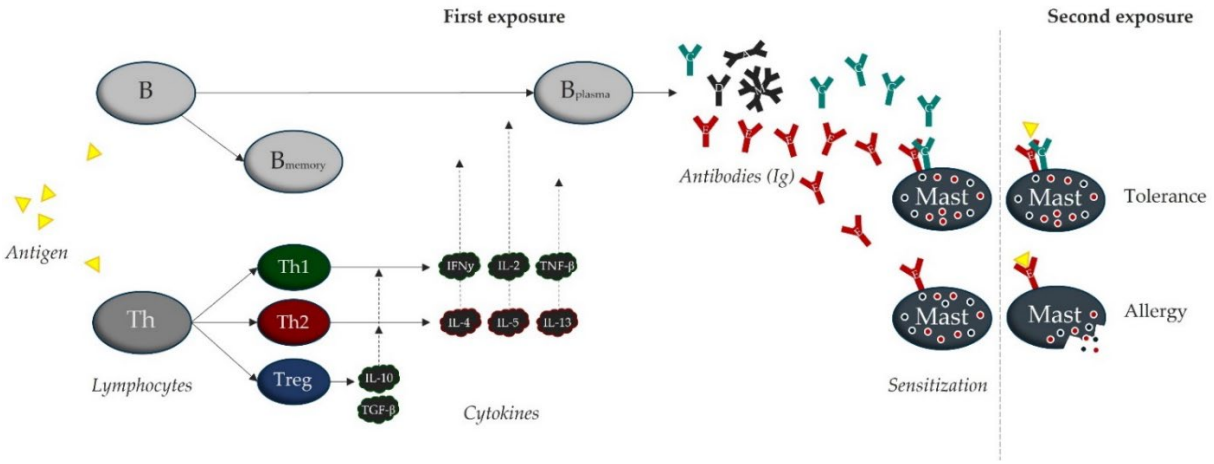

Figure 2. Overview of allergic sensitization and allergy development.
An exogenous substance (antigen) causes the immune cells (T and B cells) to mature into subsets. Depending on how the T helper cell matures, different cytokines are produced which stimulate or inhibit the plasma B cell to produce different antibodies. Immunoglobulin E (IgE) attaches to the surface of mast cells in a process called allergic sensitization. At second exposure, the antigen binds to the antigen-specific IgE which causes a release of inflammatory substances from the mast cells, resulting in allergic symptoms. *Abbreviations*: B, B cell; Th, T helper cell; Treg, regulatory T cell; IL, interleukin; Ig, immunoglobulin; Mast, mast cell.

3.1.1. Hygiene hypothesis

The prevalence of allergy has been on the rise over the last decades, and the relatively rapid change cannot be assigned to genetics [17]. Exposure to a microbe-rich milieu promotes tolerance according to the hygiene hypothesis [2], and low microbiota diversity has been suggested to precede eczema development [18]. Exactly how the microbes shape the immune system is still unknown.

3.1.2. Influence of maternal diet on offspring allergy development

The maternal diet during pregnancy and lactation can affect the fetal immune system development and, consequently, influence allergy development. The dietary components often affect the balance between the different $CD4^+$ cells (i.e., T helper cells and regulatory T cells) [19]. The research regarding maternal food intake and offspring allergy risk is rapidly evolving, although there are still large gaps of knowledge [20]. In recent years, numerous reviews have been published that touch upon the role of maternal diet in offspring allergy development [11, 21-23]. Although the systematic reviews have focused on different dietary components (e.g., whole diet, Mediterranean diet, or sugar intake), they all end up concluding that there is insufficient or limited evidence of both protective and harmful effects of maternal diet on offspring allergy development.

In order to complement the knowledge summarized in previous reviews, a systematic literature search regarding publications from the last five years was conducted during the work with this thesis. The search methodology and reasons for exclusion are presented in the **Appendix**. In total, 35 of the publications met the inclusion criteria and are summarized in **Appendix Table A1**. The results indicate that the current focus in the research field is to investigate maternal diet during pregnancy and not lactation. Further, maternal diet is often assessed with some kind of index before related to offspring allergy. Several studies seem to focus on the intake of PUFAs and antioxidants. The most common dietary assessment method seems to be a single food frequency questionnaire filled in during pregnancy. For allergy assessment, the most common approach was to ask the parents if the child ever had received a physician diagnosis (i.e., not a standardized diagnosis by the same allergologist). The distribution of studies investigating food allergy, eczema, and/or asthma was similar. The most common age for allergy assessment was during the first year of life for both food allergy and eczema, whilst no clear age pattern could be seen for asthma. Since the methodology, setting, and age differ between studies (e.g., some report point prevalence while some report cumulative incidence), the number of children with the different diagnoses varies greatly and cannot be summarized in a proper way (e.g., eczema varies between 4-34% during the first year of life in the different studies).

3.1.2.1. Dairy products intake and allergy

A systematic review published in 2020 concluded that insufficient evidence exists to conclude on the effect of maternal cow's milk intake on offspring allergy risk [22]. One original research paper, which was not included in the review, investigated maternal intake of dairy products during both pregnancy and lactation [24]. In that study, the lowest intake (i.e., <546 grams/day) during pregnancy was associated with consistently higher odds of cow's milk protein allergy compared to an intake between 546-1097 grams/day. In the most adjusted model, the highest intake (i.e., >1097 grams/day) presented lower odds of cow's milk protein allergy in comparison with an intake between 546-1097 grams/day. These associations were, however, not found during lactation.

Concerning studies published during the last five years (**Appendix Table A1**), one study reported that the consumption of dairy products 3-4 times/week during pregnancy, in comparison with ≤2 times/week, was associated with increased odds of offspring parent-reported eczema during the first year of life. However, the intake of dairy products ≥5 times/week was not significantly associated with offspring eczema. The study found no association between the intake of cow's milk during pregnancy and offspring food allergy [25]. Another study investigated yoghurt intake specifically and found a lower risk of reported eczema diagnosis between 3-6 months of age following a higher yoghurt intake during pregnancy. The association was found when comparing a lack of consumption with any consumption, consumption >3 times/week, as well as a consumption of 50 grams/week [26].

3.1.2.2. Fruit and vegetable intake and allergy

A meta-analysis that pooled the results from two studies found lower odds of eczema following a higher intake of vegetables during pregnancy [27]. One of these two studies found lower odds of eczema between 16-24 months of age following a higher maternal intake of green and yellow vegetables, and citrus fruit, but not total intake of vegetables or fruits during pregnancy [28]. The other study did not find any association between maternal intake of vegetables during pregnancy and offspring eczema or asthma at two years of age [29]. The authors of the latter article speculated that the lack of association could have been due to the relatively high consumption of vegetables in the cohort (90% consumed it ≥2-5 times/week).

Another study, not included in the meta-analysis, with data from both pregnancy and lactation found that an intake of >599 grams/day of fruit and berries was associated with increased odds of offspring cow's milk protein allergy, compared to intakes between 218-599 grams/day [24]. This association was noted during lactation, but not pregnancy. Regarding more recent literature (**Appendix Table A1**), a study conducted in Denmark indicated lower odds for offspring eczema at three years of age following a higher intake of fruit (assessed with plasma levels of stachydrine and FFQ) during pregnancy [30]. Another study, solely including children with eczema, found a lower asthma prevalence (unclear age for diagnosis, likely during the first three years of life) among children to mothers who consumed more fruit and vegetables during pregnancy [31]. On the contrary, a study conducted in China found no association between fruit intake during pregnancy and offspring eczema during the first six months of life [32].

3.1.2.3. Seafood intake and allergy

Seafood could be expected to influence allergy development due to its content of the immunoregulatory long-chain polyunsaturated fatty acids (LCPUFAs) [6, 33, 34]. A systematic review and meta-analysis including research published before February 2020, found no association between maternal fish intake during pregnancy and offspring eczema [35]. Regarding food allergy, the authors presented an inverse association between maternal fish intake and offspring food allergy [35]. This was based on five different studies, out of which two presented a significant inverse association between maternal fish consumption (type not specified in any of the studies) and offspring food allergy. Two other reviews focusing specifically on seafood intake and offspring allergy risk concluded that maternal intake during pregnancy does not seem to be associated with offspring allergy [36, 37], whilst one (including both pregnancy and lactation) suggested that fish intake might be beneficial but needs further evaluation [10].

Concerning fish intake during lactation, our research group have in a previous cohort (FARMFLORA) shown maternal intake of fatty fish during lactation to be positively associated with EPA, DHA, and n-3 LCPUFA levels in both breast milk and infant's serum phospholipids at four months. In turn, higher serum levels of EPA in infants were associated with lower odds of allergy at three years of age (OR (95% CI)=0.47 (0.27-0.83) following a 0.1% increase in the proportions). This association was found also in confounder adjusted models (e.g., allergic heredity, delivery mode) despite the low number of allergic children (N=9) [38]. Another research group investigating maternal intake of fish (type not specified) during lactation found no associations with offspring cow's milk protein allergy [24].

The more recently published studies (**Appendix Table A1**) could not clarify these conflicting results. One study reported higher odds of food allergy during the first year of life following an intake of fish 1-2 times/week, but not ≥3 times/week, in comparison with an intake less than once per week during pregnancy [25]. Another study found that mothers to children without cow's milk protein allergy more often consumed fish 2-3 times/week than mothers to children with cow's milk protein allergy (48% versus 18%, respectively) [39]. A third study could not identify any associations between maternal intake of seafood during pregnancy and offspring food allergy [31]. The different types of fish were not investigated separately in any of these studies. Original research papers from the last five years investigating fish intake during lactation were not found with the present search strategy.

3.1.3. Fatty acids and the immune system

Polyunsaturated fatty acids (PUFAs) are among the most widely studied dietary components in relation to the immune system. The theory that dietary intake of these fatty acids is involved in allergy development was raised already three decades ago [7]. At that time, an article published a theory stating that the observed rise in asthma prevalence occurred in parallel with changed food habits, and that the change in food habits therefore could explain the increased number of asthma cases [7]. The theory was further developed to include allergic sensitization in general and not only asthma [6]. According to the theory, the consumption of n-3 PUFAs (e.g., fatty fish) has been replaced with n-6 PUFAs (e.g., vegetable oils) following societal trends. As can be noted, the theory is based on observations, and the causality is still debated [40].

Both n-3 and n-6 LCPUFAs are incorporated into the membranes of the immune cells, which affects e.g., cell signaling. *In vitro* studies have showed that lipids, particularly LCPUFAs, are immunomodulatory, suppressing T cell activation and IFN-γ production through an effect on the dendritic cell/T cell interaction [33]. Studies in our laboratory, using mice, have shown that n-3 LCPUFAs downregulate the innate and adaptive immune system [34].

A meta-analysis including cohort data published between 2002 and 2014, found no associations between maternal intake of fat (total, saturated, monounsaturated, n-6 PUFAs, and n-3 PUFAs) during pregnancy and offspring eczema or asthma, referring to six different studies, out of which none looked at the association to food allergy [27]. In 2016 (i.e., two years after the scope of that review) our research group published results from the FARMFLORA study [41]. Higher odds of an allergy diagnosis at three years of age were found following a higher maternal intake of margarine and oils both during pregnancy (OR (95% CI)=1.91 (1.02-3.56) for a 5 g increase) and lactation (OR (95% CI)=1.50 (1.02-2.21) for a 5 g increase). These associations remained significant also after adjusting for delivery mode, but not allergic heredity, despite few allergic children (N=10 and N=6, respectively) [41].

3.2. Dietary assessment

In order to understand the role of diet in disease development, assessing both the exposure and the properly is crucial. There are different ways to measure dietary intake: subjective (e.g., food frequency questionnaires) and objective (e.g., food intake biomarkers in blood and urine). The subjective methods include different types of questionnaires and interviews and are commonly prone to measurement errors [12, 42]. The general difficulties with the subjective dietary assessment methods include, for instance, recall bias, social desirability bias, and interviewer bias [42]. Further, nutritional calculations are often

used to quantify the intakes of macro- and micronutrients based on the data collected with the questionnaire and/or interview [12, 42]. The quality of such calculations is highly dependent on the availability of updated and detailed food composition databases, and often the available information is not enough to fully cover variations due to e.g., season (e.g., fatty acid content in fish) or geographical origin (e.g., selenium content in soil) [12].

Regarding the objective measurements, they also have sources of errors. For instance, specificity could be a problem since the compound (e.g., blood levels of a micronutrient) often can be affected by exogenous factors other than one specific dietary source, and by endogenous synthesis or metabolism [12]. Hence, a large challenge is knowing what the biological compound actually reflects (e.g., if the body regulates the levels closely with compensatory mechanisms or if the concentrations are solely affected by food intake). In addition, laboratory practices (e.g., instrument, storage, or thawing) might affect the biological sample and, in turn, the measured concentrations [12].

Depending on the research purpose, the choice of method can vary [42]. Although there are drawbacks to consider regardless of approach, a combination of subjective and objective measurements could be useful since the sources of errors are expected to be of different natures [12].

3.2.1. Food frequency questionnaire

The use of food frequency questionnaires in epidemiological research is often the most convenient choice due to the relatively low cost and participation burden. This dietary assessment method focuses on assessing the whole diet by asking about the intake frequency of a wide range of food items. An enhanced version is often referred to as semi-quantitative and include questions about the consumed amount either by specifying regular household measures and/or by depicting different portion sizes visually. Regardless of semi-quantitative or not, the method measures the average intake over a longer period of time rather than single days (e.g., 24h recall), which might be an advantage since the within-person variation in food intake might be large between days [12]. Also, recalling the usual intakes might often be easier than recalling single intakes of a specific day [12].

3.2.2. Food intake biomarkers

Within epidemiological research, a biomarker has been proposed to be defined as “an objective measurement to assess the exposure, effect, or susceptibility of the human organism” [43]. Within this overarching definition, different subcategories can be used to describe the biomarker more in detail: exposure biomarkers, effect biomarkers, and susceptibility biomarkers [43]. Food or food component intake biomarkers can in turn be grouped under exposure biomarkers and are defined based on their ability to “measure the intake of specific food groups, foods, or food components (such as ingredients) and can be used to estimate recent or average intakes of these entities” [43]. There are several publications referring to measurable components in biological samples affected by food intake as reviewed elsewhere [44]. Importantly, the magnitude of the exposure (e.g., amounts consumed) but also the susceptibility of the specific individual can influence the concentrations measured in the biological sample [43].

In this thesis, food intake biomarkers (i.e., exposure biomarkers) were used with the aim of complementing self-reported dietary data. Further, biomarker exposures through seafood consumption were investigated (e.g., mercury and arsenic). Depending on the interpretation of the classification scheme [43], the latter biomarkers could be classified under food component intake biomarkers (i.e., if one considers the environmental pollutants to be an unavoidable seafood component), or simply as exposure biomarkers to the specific pollutants. For perspective, in the latest Swedish market basket study conducted by the Swedish Food Agency, arsenic and mercury were identified in all of the tested fish products (i.e., fish commonly bought on the Swedish market) [45].

Although food intake biomarkers could be seen as a more objective measurement of food intake, the number of validated biomarkers is limited. Criteria for validation of a food intake biomarker have recently been suggested and include eight aspects: plausibility, dose-response, time-response, robustness,

reliability, stability, analytical performance, and reproducibility [46]. In theory, a fully objective biomarker should solely be affected by the intake of one specific food item (or food group, if that is the purpose) and not be affected by the intake of other food groups. Further, the biomarker should not be endogenously produced. However, in reality, the diet consists of several different foods, and one never consumes only one isolated food item. So, even though the desired food intake biomarker is affected by several different food sources, it can still reflect the intended food item if the other sources affect the concentrations to a lower extent (or are more rarely consumed). In addition, since diet comprises food items that are highly intercorrelated, also purely isolated metabolites known to be affected solely by a specific food item could be associated with the reported intake of other foods [12].

3.2.2.1. Biomarkers of dairy product intake

Dairy products are a heterogeneous food group and comprises a wide range of food items such as fermented (e.g., cheese) and raw cow's milk (e.g., pasteurized cow's milk, low-fat cow's milk). Literature often suggests the use of the saturated fatty acids pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) as dairy product intake biomarkers [47, 48], and commonly relate these concentrations to e.g., heart disease [49] and type 2 diabetes [50].

These specific fatty acids are produced by microbial fermentation in the cow's rumen and are, therefore, often considered as biomarkers of dairy intake [48]. However, a randomized controlled trial indicated that also humans can produce these fatty acids endogenously from propionate, a short-chain fatty acid produced from gut microbiota fermentation of dietary fibers [51]. Importantly, they therefore highlight that health outcomes (e.g., type 2 diabetes) associated with these fatty acids might partly be confounded by the intake of dietary fiber. However, regardless of the outcome, several studies have reported C15:0 and C17:0 levels in different types of biological samples to be associated with the reported intake of dairy products [47, 52].

Until better dairy intake biomarkers are available, it is important to remember that these fatty acids are not fulfilling the demands (e.g., specificity) for being validated as food intake biomarkers [47]. As reviewed elsewhere, C15:0 commonly correlates more strongly to self-reported dairy intake than C17:0, regardless of lipid fraction and type of sample [47, 48]. It must be considered that blood levels might be affected by endogenous production and by the intake of other food sources, such as fish, although the concentrations are much lower [53].

3.2.2.2. Biomarkers of seafood intake

Seafood is a heterogenous group both in terms of nutrient composition (e.g., fatty fish versus lean fish) and also in terms of environmental pollutant content (e.g., depending on catchment area). As reviewed elsewhere, the most frequently used seafood intake biomarkers are the n-3 LCPUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [54]. The measurement of these fatty acids can be effective in reflecting a habitual intake of fish, primarily fatty fish, since the steady state of these is reached first after two to six weeks (plasma) and four weeks to six months (erythrocytes) [54].

Further, the furan fatty acid 3-carboxy-4-methyl-5-propyl-2-furanepropanoic acid (CMPF) is mentioned in several publications, including a randomized controlled trial which suggested CMPF to be highly specific for fatty fish intake [55]. However, studies investigating shellfish (i.e., not fatty fish) biomarkers have also included CMPF as a food intake biomarker [54]. Concerning lean fish, most studies have measured trimethylamine oxide (TMAO) in urine [54], although the metabolite can be produced endogenously from the intake of other animal food products [56]. There are also studies on lean fish that have measured arsenobetaine and other arsenic compounds, as well as DHA and EPA [54].

In summary, the literature presents several different candidate seafood intake biomarkers, with EPA and DHA fulfilling the validation criteria as thoroughly reviewed in the FoodBAll project [54]. Important to note is that studies rarely include pregnant or lactating women. The plausibility of using food intake biomarkers for pregnant women, validated in a non-pregnant population, is uncertain [57]. Importantly,

the n-3 LCPUFA DHA is readily transported over the placenta for accumulation in the fetal brain and tissues [58], and levels in maternal samples might, therefore, not only reflect her intake. Furthermore, endogenous production of LCPUFAs is enhanced in women of fertile age [59, 60].

3.3. Food intake during pregnancy and lactation

Pregnancy is a period in life when food intake is no longer solely needed for the maternal nutritional status but also for the growing fetus. Pregnancy can be divided into three trimesters (≤13 weeks, 14-26 weeks, and >26 weeks of gestation). Most fetal growth occurs during the second half of the pregnancy [61], and the additional need for energy in the last trimester deriving from the diet is around 450 kcal/day [62]. Some micronutrients are crucial for fetal, infant, and maternal health, and the recommended daily intakes are, therefore, higher compared to non-pregnant or lactating women. For instance, adequate intake of folate is known to drastically lower the risk of neural tube defects which has led to higher recommended intakes for all fertile women [63]. Commonly, the increased micronutrient need is even higher during lactation due to the production of breast milk and the excretion of the micronutrient via breast milk (e.g., recommended daily intake of folate increases with 100 µg/day from pregnancy to lactation) [63]. In addition to folate, micronutrients mentioned as of special concern during these life phases include, for instance, iron (e.g., due to increased red blood cell count and expected blood losses at delivery) [64], as well as iodine and selenium (e.g., combined deficiency have been inversely associated with neurodevelopment) [65]. Also, the recommended contribution of PUFAs, with an emphasis on DHA, to the total energy intake is increased [63].

Although there is an increased need for energy and micronutrients during both pregnancy and lactation, several mechanisms in the human body can to some extent compensate for this (e.g., reduction in maternal storage) [66]. Hence, although the estimated need for a micronutrient is increased, this does not automatically imply a need for increased dietary intake considering the compensatory mechanisms (e.g., increased absorption, bone resorption, and renal conservation) [63]. The Nordic Nutrition Recommendations, which form the basis for dietary guidelines in Scandinavian countries, are currently being updated. Therefore, it is possible that some of the recommendations mentioned in this section will change after the publication of this thesis.

3.3.1. Nutrient transport to the fetus

For the fetus to grow, oxygen and nutrients must be transported from the maternal circulation to the fetus via the placenta. The placenta is a highly advanced organ that regulates the transportation of nutrients from the maternal circulation to the fetal circulation, and vice versa, as well as filtering some components by storing them in the tissue. The efficiency of nutrient transport is affected by several factors, including the size of the placenta and the location and number of specific transport proteins [67]. The primary transportation of nutrients involves glucose, fatty acids, and amino acids, which all provide energy and building blocks for the growing fetus [68].

Glucose is transported from maternal circulation by facilitated diffusion, involving glucose transport proteins [68]. Fatty acids are transported across the placenta as free fatty acids via specific transport proteins [68]. Amino acids are transported from maternal circulation to the placenta via several different transport systems and then further transported into fetal circulation with facilitated diffusion involving transport proteins [68].

3.3.2. Provision of nutrients to the infant

After birth, infants are fully dependent on parental feeding either with breast milk or formula. The breast milk is sufficient to support the infant with both micro- and macronutrients, except for vitamin D and vitamin K, which are routinely given in the form of fat drops to all children under two years of age, and as an injection to the newborn, respectively. To investigate the influence of maternal diet on the infant's immune system during lactation, transportation of nutrients and immunoregulatory factors via breast milk is in focus.

Constituents of breast milk vary over time, with colostrum being the first milk produced in the mammary glands, followed by transitional and then mature breast milk. What is common for all different phases is that the proportion of carbohydrates (i.e., lactose) is highest, followed by fats and then proteins [69]. Immunoregulatory factors in breast milk include a wide range of immune cells, cytokines, growth factors, immunoglobulins, and human milk oligosaccharides. However, the breast milk's content differs largely between individuals depending on the time of day, diet, and for instance, if the child is born prematurely [69].

4. MATERIALS AND METHODS

4.1. Study population

The thesis is based on data from the Nutritional impact on Immunological maturation during Childhood in relation to the Environment (NICE) cohort, located in the north of Sweden (65.3–66.4°N), within the catchment area of Sunderby Hospital in Norrbotten Region. Families with a planned delivery at the hospital were approached at their first visit to maternity clinics (gestational week 10-12), with oral and written information about the study. Later on, at a routine ultrasound in gestational week 18-20, the families received more detailed, written information about the study. They were further instructed to send in written consent by post if they were interested in participating. Recruitment took place between February 2015 and March 2018.

The recruitment demanded that the families who wanted to participate in the cohort send in their consent by post without any reminder. This could explain why solely 10% of the intended population participated in the study [70]. Further, this might have led to the fact that, among the invited families, those interested in allergy and research were more eager to send in their consent to participate. The investigated children could therefore represent a high-risk (in terms of allergy) population rather than a general population. As published elsewhere, the included women were older (31 years versus 29 years), more highly educated (69% versus 43% with >12 years of education), and more often identified themselves as Swedish (94% versus 78%), in comparison with women who did not participate in the study but who gave birth at the same hospital during the same period of time [70]. They also reported consumption of supplements more frequently and were less likely to smoke. Hence, the generalizability of our findings to a general population in Sweden is not possible.

4.2. Study design

The prospective birth cohort NICE (ClinicalTrial.gov: NCT05809479) was started with the aim of clarifying the effect of environmental exposures (e.g., diet) during pregnancy and early life on the infant's immune system and allergy development. The cohort is a result of a collaboration including research groups from Gothenburg (Chalmers University of Technology, and Gothenburg University), Stockholm (Karolinska Institute), Umeå (Umeå University), as well as clinicians at the Sunderby Hospital. The cohort has been approved by the Regional Ethical Review Board in Umeå, Sweden (2013/18-31M).

This thesis focuses specifically on assessing the diet of pregnant and lactating women, using subjective and objective measurements, and their association with offspring allergy diagnosis at 12 months of age. In all papers, twins, and double-participants (i.e., families participating with a second child) were excluded. Handling these dependencies was considered unnecessarily troublesome when the number of participants it concerned was low (N=3 twin-pairs and N=16 double-participants) in relation to the whole cohort. Further, to handle unrealistic outliers, the limit of energy intake was set to 500-4000 kcal per day, and those reporting an intake outside this range were excluded.

In **Paper II** and **Paper V**, the associations concerning samples from the child only included breastfed infants with the reasoning that maternal diet could solely influence the infant via breast milk. An overview of the included subjects, investigated outcome, objective, and hypothesis of each paper is presented in **Table 2**.

Table 2. Overview of the papers included in this thesis.

	Paper I	Paper II	Paper III	Paper IV	Paper V
Subjects	• Pregnant women	• Pregnant women • Lactating women • Breastfed infants	• Women during delivery • Fetus (umbilical cord)	• Pregnant women	• Pregnant women • Postpartum women • Breastfed infants
Exposures	Maternal characteristics	Maternal diet	Fatty acid in plasma phospholipids, maternal diet	Seafood intake	Maternal diet
Outcome	FFQ reported food intake (g/day)	Offspring allergy diagnosis at 1 year	Offspring allergy diagnosis at 1 year	Seafood biomarkers	Plasma metabolites
Sample size	567	508[1]	206[2]	554	193-579[3]
Thesis objective[4]	a	b, c	b	c	c
Hypothesis	Lifestyle and characteristics affect food choices	Food intake during pregnancy and lactation affect offspring allergy risk	Fatty acid profile *in utero* affects allergy risk during the first year of life	Reported seafood intake can be reflected with more objective measurements in both blood and urine	Reported food intake can be reflected with objective biomarker measurements in blood plasma

[1] Number of children with allergy diagnosis included in correlation analyses concerning pregnancy, 1 month, and 4 months: eczema, N=32, N=31, N=27; food allergy, N=38, N=34, N=30; and asthma, N=31, N=26, N=24.
[2] Number of children with eczema diagnosis, N=14.
[3] Number differs depending on sampling occasion and were as follows: pregnancy: N=579; delivery: N=523 (mothers) and N=348 (children); 4 months postpartum: N=477 (mothers) and N=193 (children).
[4] Thesis objectives: **a)** investigate maternal characteristics and lifestyles associated with dietary intake during pregnancy, **b)** investigate if dietary intake during pregnancy and lactation is associated with offspring allergy, and **c)** explore candidate food intake biomarkers during pregnancy and lactation in relation to diet and offspring allergy, applying targeted biomarker analyses and metabolomics.

4.3. Data collection

The data was purely observational. All data were collected using questionnaires, except for allergy diagnosis and biological sampling. An overview of the data (including biological samples) collected for the different papers is presented in **Figure 3**. Information regarding reported food intake during pregnancy was used in all papers of this thesis. Biological samples were used in all papers except **Paper I**.

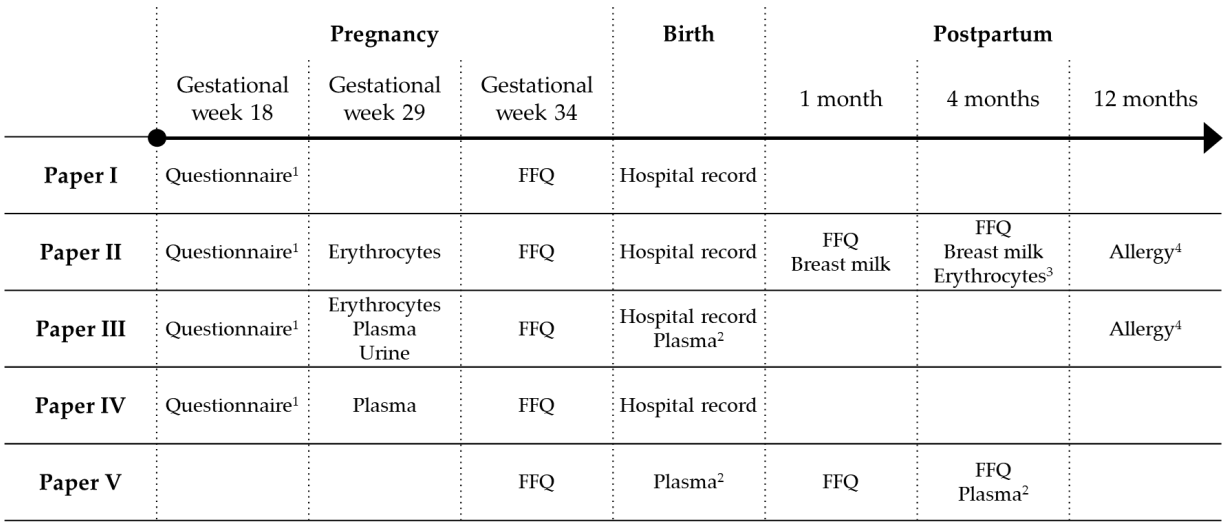

Figure 3. Overview of data and sample collection in the NICE cohort used in this thesis.
Abbreviations: FFQ, food frequency questionnaire.
[1] Including questions regarding e.g., sociodemographic factors, home environment, and allergies. [2] Mother and child (umbilical cord). [3] Mother. [4] Physician's diagnosis of the child.

4.3.1. Dietary assessment

Dietary data were collected using a repeated web-based semi-quantitative food frequency questionnaire (FFQ) sent out by email in gestational week 34, one month postpartum, and four months postpartum. The women were asked to report their intake during the last month. The specific FFQ, Meal-Q, was developed by an independent research group at Karolinska Institutet, Sweden [71, 72]. The Meal-Q includes 102-174 questions, where individuals reporting certain intakes were asked follow-up questions while those who

did not report any consumption were not given any further questions regarding that specific food item (e.g., soft drinks, sugar or sweeteners). The questionnaire is semi-quantitative and intakes in grams per day were calculated based on reported intake frequency in combination with normal portions as presented in the Swedish Food Composition Database provided by the Swedish Food Agency (**Paper I**) [73], and further personalized with pictures of portion sizes in the Meal-Q (**Paper II**-**Paper V**).

The original Meal-Q was slightly modified before the use in our cohort. The modifications included additional details regarding fat content in dairy products, as well as distinguishment between sugar sweetened and artificially sweetened beverages. Also, a distinguishment between crushed and intact flaxseeds was included by adding follow-up questions for participants reporting any consumption of flaxseeds. These extra details were included in the nutritional calculations of the whole diet. Further, not affecting the nutritional calculations, more detailed questions were added regarding game meat and lamb, consumption of fish before versus during pregnancy, use of organic food products, use of probiotic products, and avoidance of gluten and lactose [74].

4.3.1.1. Methodological considerations for dietary assessment

Since the cohort involved over 650 families, a FFQ was chosen over more invasive and personnel-requiring methods such as a 7-day weighted food record or repeated 24h recalls. Regarding the use of Meal-Q in particular, two articles concerning the validity of the questionnaire have been published by the team which developed the method [71, 72]. The validation study, called VALidation of Methods Assessing diet and physical activity (VALMA), included N=180 healthy adults in Stockholm, Sweden. The participants in the validation study were, on average, 33 years and highly educated (80% with >12 years in school). Further, 29% of the participants studied or worked with nutrition. It is important to note that pregnant and breastfeeding women (more specifically, women who gave birth within ten months before the study) were excluded from the validation study. All participants were divided into three groups distributed by age and gender. All groups responded to the web-based Meal-Q (once by the first group and twice by the second and third groups, with three weeks in-between), and did also fill in a web-based 7-day weighted food record which acted as a reference method (participants were provided with a household scale). Misreporters of energy intake, based on the weighted food record in comparison with the energy expenditure (all groups reported number of steps and physical activities, and the third group also received doubly labeled water), were excluded applying the Goldberg cut-off (i.e., comparing the ratio between reported energy intake and estimated basal metabolic rate with physical activity level) [75].

The first publication from the validation study focused on energy intake and macronutrients. It showed that the reported intake of energy and macronutrients were significantly lower with the Meal-Q compared to the 7-day weighted food record, except for PUFAs, which did not differ [72]. The intake reported with Meal-Q covered 83% of the energy, 93% of the protein, 87% of the carbohydrates, and 76% of the total fat reported with the reference method. The same trend (i.e., lower levels in Meal-Q reported intake compared with weighted 7-day food record reported intake) was seen in the second study regarding micronutrients and fiber intake [71]. However, the authors concluded that the Meal-Q is useful for ranking intakes of dietary fiber and all micronutrients, except sodium [71]. Further, the reproducibility was found to be good concerning energy, macronutrients, and micronutrients. They concluded that the Meal-Q was user-friendly, had a short answering time (mean 17 minutes), and provided sufficient information for meaningful ranking of the food intake [72].

Taking the semi-quantitative nature, the adjustments made to our cohort, and the results from the validation study into account, the method was considered time-efficient, cost-effective, as well as reasonable accurate for this cohort. However, as mentioned above, the Meal-Q has not been validated for pregnant or breastfeeding women. Furthermore, the validation study shows a systematic tendency for underreporting which must be kept in mind when interpreting the absolute intakes in the NICE cohort.

4.3.2. Maternal characteristics

Information regarding maternal characteristics was collected with a questionnaire sent out in gestational week 18 (e.g., education, residential area), extracted from the medical records (e.g., parity and BMI), and retrieved from interviews conducted at the study visits (e.g., family history of atopic disease).

4.3.3. Biological samples

An extensive collection of biological samples was done in the NICE cohort, including feces, meconium, placenta, umbilical cord, urine, blood, saliva, and breast milk. Samples were collected from the mothers, fathers, and their children. More detailed information can be found in the study protocol [76]. For this thesis, samples from mothers and children were used and included umbilical cord blood, urine, venous blood, and breast milk.

Blood from the mothers was drawn from the cubital vein and collected in 10 mL EDTA tubes (Becton Dickinson, New Jersey, USA) for later fatty acid analysis, and in 6 mL trace element free Na-heparin tubes (Greiner bio-one, Kremsmünster, Austria) for trace element analyses. Sampling during pregnancy was done at the different local maternity clinics. Sampling during the delivery was done at the hospital by the midwives at the delivery ward, and postpartum at follow-up visits at the study center by the study nurses. Mid-stream, spot urine samples were collected in polypropylene cups (Sarstedt Inc, North Carolina, USA), and later transferred to trace element free 24 mL polyethylene bottles by the study personnel. Breast milk was collected in 15 mL polypropylene tubes (Sarstedt Inc, North Carolina, USA). The women were instructed to collect the breast milk in relation to a breastfeeding occasion occurring before noon.

4.3.4. Allergy diagnosis

Allergy during the first year of life was diagnosed at 12 months of age by the study pediatrician specialized in allergology. In total, 539 children attended the clinical follow up at one year. Out of these, 36 children were diagnosed with eczema, 43 with food allergy, and 35 with asthma. Hence, the incidence of physician diagnosed eczema, food allergy, and asthma were 6.7%, 8.0%, and 6.5%.

Food allergy was diagnosed based on reported allergic symptoms after provocation at home of a specific food item, which had previously been causing symptoms from the same organ and led to improvement after avoidance. If a previous exposure to a specific food allergen was reported to cause an acute severe reaction, provocation was not deemed necessary for confirmation and hence not encouraged.

Williams criteria were used to diagnose atopic eczema [77-79]. As stated in the criteria, mandatory for diagnosis is parent-reported itchiness (manifesting as scratching or rubbing by the child) in combination with ≥3 of the following criteria: 1) involvement of skin creases (e.g., elbows, knees, ankles, neck, or cheeks), 2) history of asthma or hay fever, or history of atopic disease in a first-degree relative, 3) history of general dry skin, 4) visible eczema in flexural areas, cheeks, forehead, or outer limbs.

Early-life asthma was diagnosed if the child had wheezing between infections or persistent for at least four weeks, three episodes of wheezing during infection or, if concurrent allergic disease, any wheezing during infection.

4.4. Laboratory analyses

Data from laboratory analyses were used in all papers of this thesis except for **Paper I**. Analyses were primarily conducted using chromatography for the separation of the desired compounds and mass spectrometry (MS) for the quantification and identification of the compounds. Fatty acid proportions were analyzed in plasma phospholipids (**Paper III**), erythrocytes (**Paper II** and **Paper IV)**, and breast milk (**Paper II**). Concentrations of trace elements (**Paper IV**) were analyzed in 1) urine: total arsenic, inorganic arsenic metabolites, arsenobetaine, arsenocholine, trimethylarsine oxide, and iodine, 2) plasma: selenium, and 3) erythrocytes: mercury, total arsenic, and selenium. Metabolomics were used to analyze metabolites in blood plasma (**Paper V**). Identification of the metabolites was conducted by 1) comparing the MSMS

spectra with library spectra, and 2) using the SIRIUS Software [80] to find probable structures based on mass-to-charge ratio and retention time. More detailed information regarding the laboratory methods can be found in the different papers.

4.5. Statistical analyses

The dietary data were non-normally distributed, which narrowed the suitable methods to the non-parametric ones. With the objective to investigate associations between diet and allergy, keeping an exploratory approach, correlation analysis was selected and used in four out of five papers (**Table 3**). The correlation analyses enable presentation of results using heatmaps, which can be favorable when having several exposures and outcomes. Further, to get an effect size, logistic regression was used in **Paper III** (i.e., the paper without correlation analysis).

Univariate statistics were used in all papers and included Pearson's Chi-square, Fisher's exact test, Mann-Whitney U, Kruskal-Wallis test, and Spearman correlation. For the papers involving allergy as an outcome, multivariate statistics were used in the form of partial Spearman correlation, principal component analysis (PCA), partial least squares projections to latent structures (PLS) regression, and orthogonal PLS (OPLS-DA) (**Table 3**).

Table 3. Statistical analyses used in this thesis.

	Paper I	Paper II	Paper III	Paper IV	Paper V
Purpose and tests	• Visualize food intake versus characteristics - Spearman correlation - Unsupervised hierarchical cluster analyses - PCA • Food intake vs characteristics - Mann-Whitney U - Kruskal-Wallis	• Characteristics differences (allergic vs non-allergic) - χ^2 - Fisher's exact test • Visualize food intake versus allergy - Unsupervised hierarchical cluster analyses - Partial Spearman correlation	• Characteristics differences (allergic vs non-allergic) & proportions fatty acids differences (mother vs child) - χ^2 - Fisher's exact test - Mann-Whitney U • Proportions fatty acids (allergic vs non-allergic) - Logistic regression - OPLS-DA • Food impact on blood levels of fatty acids - PLS	• Normality check of food intake and biomarkers - Shapiro-Wilk test • Characteristics and biomarker concentration differences (high vs low seafood intake) - Mann-Whitney U - Fisher's exact test • Food intake and biomarker concentration - Spearman correlation - Unsupervised hierarchical cluster analyses - Scatter plot with Lowess smoothing lines	• Feature versus food intake - Spearman correlation - Unsupervised hierarchical cluster analyses
Software	• IBM SPSS ver. 25[1] • R ver. 3.5.1[2] • SIMCA Umetrics ver. 15.0.2[3]	• IBM SPSS ver. 26 • R ver. 3.6.2	• IBM SPSS ver. 27 • R ver. 3.6.2 • SIMCA Umetrics ver. 16.0.1	• IBM SPSS ver. 28 • R ver. 3.6.2	• R ver. 3.6.2
Covariates	-	Allergic heredity, siblings, season of birth, total energy intake	Allergic heredity and FLG loss-of-function mutation	-	-
Multiple testing adjustment	• α set to 0.001 • Univariate solely for rho>0.1 and rho<-0.1	-	• VIP>0.8 • Univariate solely for strongest associations	-	-

Abbreviations: PCA, principal component analysis; χ^2, Pearson's Chi-square; OPLS-DA, orthogonal partial least squares (discriminant analysis); PLS, partial least squares projections to latent structures (regression); FLG, filaggrin; VIP, variable importance (influence on projection).

[1] IBM SPSS (IBM, New York, NY, USA)

[2] R Studio (R Foundation for Statistical Computing, Vienna, Austria)

[3] SIMCA (Sartorius Stedim Data Analytics AB, Umeå, Sweden)

5. RESULTS AND DISCUSSION

This thesis investigated diet and dietary biomarkers during pregnancy and lactation in relation to offspring allergy development. The results showed that the self-reported food intake during pregnancy correlated with a wide range of maternal characteristics, such as age and education level. The intake of different food groups was kept fairly constant from pregnancy until four months postpartum, although the intake of dairy products and fruit and berries gradually decreased. Regarding the relationship between diet and allergy, the results show an inverse association between offspring food allergy and maternal intake of cow's milk (fresh, pasteurized) and dairy products (e.g., yoghurt and cheese) during lactation and an inverse association between all investigated allergies and maternal intake of saturated fat during lactation. A positive association was identified between n-6 PUFAs in umbilical cord plasma phospholipids and eczema incidence.

5.1. Maternal characteristics associated with food intake during pregnancy

The results in this thesis show several characteristics to be associated with the food intake in this cohort of pregnant women in Northern Sweden. For instance, age and education level were associated with food choice, where older and more highly educated women reported higher consumption of fruits and vegetables and less fast food than younger and less educated women. Further, a higher BMI and tobacco were associated with consuming less fruit. Also, the period of life (e.g., pregnancy versus postpartum) appears to be related to food choices. In summary, food intake during pregnancy is interlinked with several lifestyle factors, which makes it difficult to determine the specific significance of food intake *per se* in relation to allergy outcomes.

As can be seen in **Paper I**, the most pronounced differences in food intake were found to be age, education, BMI, and smoking tobacco before pregnancy. Living in a more rural area was related to a higher intake of meat products, game meat in particular, but also cow's milk. The literature on the impact of residential areas on food intake in Sweden is sparse. One study involving adolescents in West Sweden found that individuals living most urban consumed fruit, vegetables, and fish more frequently than those living in a more rural area [81]. That study cannot confirm our findings regarding meat and cow's milk intake since this was not investigated. However, in our study, vegetable and fruit intake clustered on the opposite side of meat and cow's milk, and thereby also of residential area (**Paper I**).

Further, not all food items were related to maternal characteristics. For instance, neither the intake of whole grain products, fiber-rich carbohydrate sources, nor lean fish differed between women of different ages or with different educational backgrounds. However, when investigating seafood intake with median split (i.e., comparing relatively high seafood consumers with relatively low seafood consumers), the educational background did differ between the consumer groups (**Paper IV**).

The food intake did not only differ depending on maternal characteristics such as education and age, we also show that reported intake differed between pregnancy, one month postpartum, and four months postpartum (**Paper II**). There was a clear gradual decrease in the reported intake over time of dairy products (310 grams/day, 270 grams/day, and 230 grams/day) and fruit and berries (270 grams/day, 200 grams/day, and 170 grams/day). In addition, the pattern of seafood consumption seemed to differ from before pregnancy and during pregnancy, where fish known to be dense in e.g., heavy metals (e.g., fish from the Baltic Sea) were consumed at a markedly lower rate during pregnancy than before (**Paper IV**).

5.2. Influence of maternal diet on infant allergy development

This thesis presents results based on associations between self-reported food intake and physician diagnosis of food allergy, atopic eczema, and asthma at 12 months of age (**Paper II**). Several statistically significant associations were found between self-reported intake of food items, as well as nutrients from nutritional calculations of the whole diet, and offspring allergy diagnosis (**Figure 4**). As can be seen in the

figure, most associations were found for maternal food intake between three and four months postpartum, rather than during pregnancy or the first month postpartum.

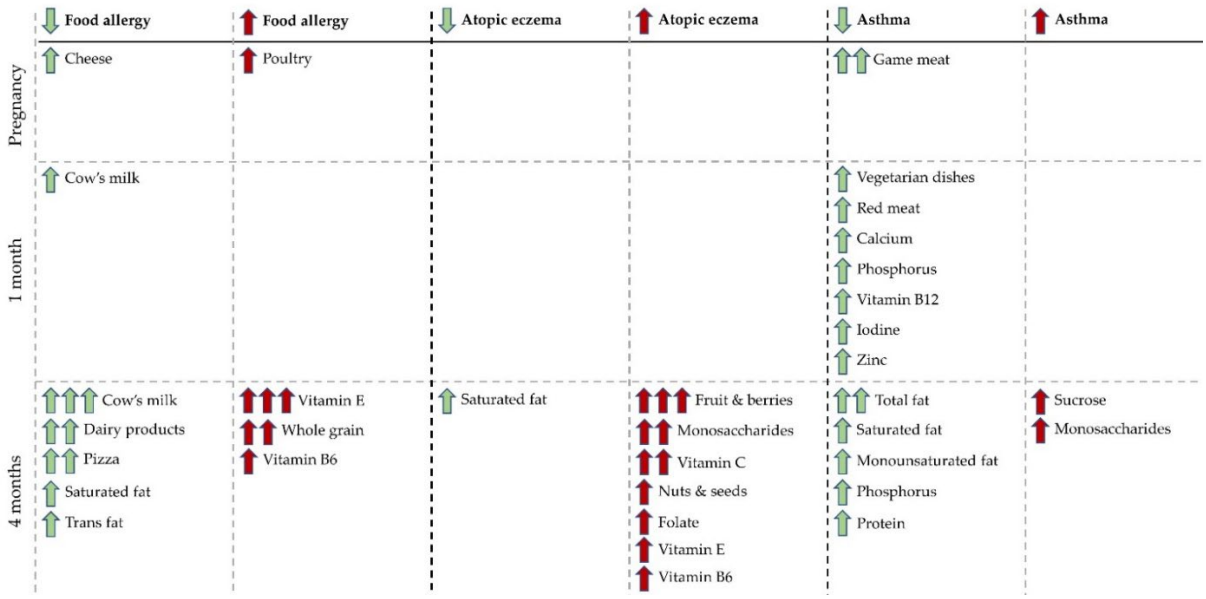

Figure 4. FFQ reported food intake associated with allergy diagnosis in the NICE cohort.
The number of arrows displays the level of significance received in the partial Spearman correlation analysis adjusted for any allergy within the family, siblings, season of birth, and total energy intake. Three arrows indicate a p-value <0.001, two arrows p-value <0.01, and one arrow p-value <0.05. The results are summarized from six heatmaps presented in **Paper II**.

5.2.1. Cow's milk intake and food allergy

The most consistent finding (i.e., remaining over time) in **Paper II** was a lower incidence of food allergy following a higher intake of cow's milk (in a glass or on a plate) by the mother. A previous study identified lower levels of cow's milk protein-specific (β-lactoglobulin and casein) IgA in the breast milk of women excluding cow's milk from their diet, and lower levels of these IgA have, in turn, been found to associate with an increased risk of offspring cow's milk protein allergy [82]. Hence, a possible explanation for the allergy protective effect could be that the child receives tolerogenic antibodies toward cow's milk via breast milk.

It is important to note that although women to children with food allergies consumed less cow's milk than women to children without any allergy (median: 0 grams/day versus 43 grams/day, p<0.001), there were still over 50% of the women with a non-allergic child reporting an intake below 50 grams/day. In the whole cohort, the reported intake of dairy products decreased gradually from pregnancy until four months postpartum (310 grams, 270 grams, and 230 grams/day), whilst the median intake of cow's milk (i.e., served in a glass or plate) was kept constant between pregnancy and one month postpartum (100 grams/day) but decreased at four months postpartum (43 grams/day). This could be a sign of reverse causation since the lower intake of cow's milk by the mother might result from early allergic symptoms of the breastfed infant (i.e., symptoms developing before exposure rather than exposure leading to symptoms). As described in **Paper II**, to take reverse causation into account, secondary analyses were performed without women who changed their intake of dairy products from any to null following early allergic symptoms of the child. The results indicated a lower incidence of food allergy following a higher intake of cow's milk even after excluding these women.

Further, the results from **Paper I** indicated that women living in a more rural area consumed more cow's milk. This could indicate that intake of cow's milk is a proxy for living area, and that the hygiene hypothesis (exposure to microorganisms) can be implemented and explain the association with lower allergy incidence, and not the intake *per se*. However, only four families lived on a farm with animals (none of these children had any allergy), and conclusions are therefore difficult to draw. Milk intake was

further negatively associated with education and positively associated with parity meaning that women with higher education and mothers being first-time pregnant drank less milk.

It is possible that exposure to cow's milk protein might protect against cow's milk protein allergy specifically, due to induced tolerance after exposure to the potential allergen (e.g., β-lactoglobulin and α-S1-casein) [83]. In this cohort, children without maternal food allergy were less frequently diagnosed with cow's milk protein allergy if the mother consumed cow's milk already during pregnancy compared to women without this consumption (3% versus 9%, p=0.04). However, this must be interpreted with caution due to the low number of children with cow's milk allergy in each consumption group (N=8 and N=6, respectively).

5.2.2. Fruit and berries intake and eczema

Maternal intake of fruit and berries at four months postpartum was found to be positively associated with offspring eczema. Previous studies have rather found opposite results, with a lower risk of atopic eczema following a higher intake of fruit and vegetables during pregnancy and lactation, as reviewed elsewhere [10]. However, one previous study presented higher odds of offspring eczema at 12 months of age following a higher maternal intake of resistant starch during pregnancy [84]. To note, that study investigated different dietary fiber intakes during pregnancy and not lactation and could not see any difference in total fiber intake deriving specifically from fruit between mothers to children with and without eczema. Hence, we have not found any obvious explanation to how maternal intake of fruits and berries may increase the risk of offspring eczema, indicating that the association may be driven by confounding factors. To investigate the association found in **Paper II** further, we searched for fruit intake biomarkers using metabolomics in **Paper V**. Results from these analyses are presented in section 5.4.2.

5.2.3. Fatty acid intake and allergies

A higher maternal intake of saturated fat while breastfeeding four months postpartum was associated with a lower incidence of food allergy, eczema, and asthma (**Figure 4**). In secondary analyses, the intake of saturated fat was divided into tertiles (≤23, >23 to ≤33, >33 grams/day), where the first tertile was used as a reference in logistic regression analyses. Also, in these analyses, a higher intake while breastfeeding was associated with lower odds of food allergy in the second and the third tertile (OR (95% CI)=0.18 (0.06-0.54), and 0.29 (0.12-0.71), respectively). This was also true for eczema and asthma when comparing the highest with the lowest tertile (OR (95% CI)=0.33 (0.12-0.91), and 0.33 (0.11-0.96), respectively). The intake of saturated fat (nutritional calculations of whole diet) during lactation was most strongly associated with the reported intake of cheese (rho=0.61), followed by sweets (rho=0.54), and dairy products (rho=0.47). Previous literature regarding saturated fatty acid intake while breastfeeding is sparse, but the intake of saturated fat during pregnancy (i.e., not lactation) has been found to associate with a lower risk of asthma at five years of age [85].

To note, the maternal intake of fatty acids during pregnancy was not associated with any of the investigated allergies. This is similar to another study with a larger sample size, where no associations between maternal fatty acid intake and offspring risk of cow's milk protein allergy at three years were identified [86]. However, when stratifying for maternal heredity in that study, higher maternal intake of α-linolenic acid was associated with lower odds of offspring cow's milk allergy among children without allergic mothers [86].

5.2.4. Ethical aspect

The potential a diet regimen can have in allergy development must, of course, be interpreted in relation to the overall risk of allergic disease following allergic heredity and other environmental exposures (e.g., tobacco). If diet indeed influences the risk of allergy, changing the diet is more easily achieved than changing the genes or exposing the infants to more microbes and infections. Regardless of the results, encouraging allergic mothers to consume food they cannot tolerate should, of course, not be done.

5.3. Investigated food intake biomarkers in the NICE cohort

In **Paper IV**, biomarkers of seafood were investigated based on laboratory quantifications of trace elements and fatty acids. The work in **Paper V** did not focus on a specific food group, instead, different candidate biomarkers were measured with untargeted metabolomics and then related to the intake of corresponding food items.

5.3.1. Metabolomics-based food intake biomarkers

In **Paper V** we searched for a panel of metabolites in plasma, reflecting different food groups, based on mass-to-charge ratio and retention time in a dataset obtained after running untargeted metabolomics. The choice of metabolites to search for was based on a list of candidate food intake biomarkers created by the Chalmers Mass Spectrometry Infrastructure (CMSI). As described in **Paper V**, the list was created based on a literature survey and communication done as part of the Food phytochemicals matter for cardiometabolic health (FOODPHYT) project under the joint programming initiative a Healthy Diet for a Healthy Life [87].

The most statistically significant associations ($p<0.001$) from **Paper V**, between food intake and plasma metabolites, are summarized in **Figure 6**. The metabolites which correlated most strongly with self-reported food intake were proline betaine, pipecolic acid, CMPF, indole-3-lactic acid, and acetylcarnitine. The results regarding juice intake and proline betaine levels in maternal plasma (pregnancy: rho=0.38; delivery: rho=0.23; four months: rho=0.41) are similar to the magnitude reported in another Swedish cohort study using FFQ data (rho=0.34 for citrus and not juice) [88]. On the other hand, the correlation coefficient between fatty fish intake and CMPF in maternal plasma at four months was weaker in our study (rho=0.30) compared to the study mentioned above (rho=0.45). As seen in **Figure 6**, relative intensities of CMPF correlated most strongly with fatty fish, as can be expected, followed by other seafood, fruits, and vegetables.

The food intake biomarkers were found to be associated not only with expected food intake variables but also with a wider range of unexpected food sources. One possible explanation is that the consumption of various food items is often closely related. Food items are usually consumed in various combinations rather than separately, as indicated by the clustering feature in the figure, as well as the results presented in **Paper I**. Therefore, it is important to interpret any associations found with caution if they are not expected. If the literature does not suggest a particular food source as a means of exposure, it may be a proxy for lifestyle or, simply, a spurious finding.

As can be seen in **Figure 5**, most associations were found four months postpartum, followed by pregnancy, and lastly, delivery. This may indicate that food intake biomarkers can be measured more accurately during lactation than during pregnancy and delivery. However, it may also relate to the sample handling in the NICE cohort, which differed between the different investigated time points. At four months, samples were drawn at the research laboratory. During delivery, samples were collected at the delivery ward, and during pregnancy at various maternity clinics. Since the samples were aliquoted and frozen at the research laboratory, the time until handling was shorter and more controlled at four months postpartum (since no transportation was needed), which could have affected the results.

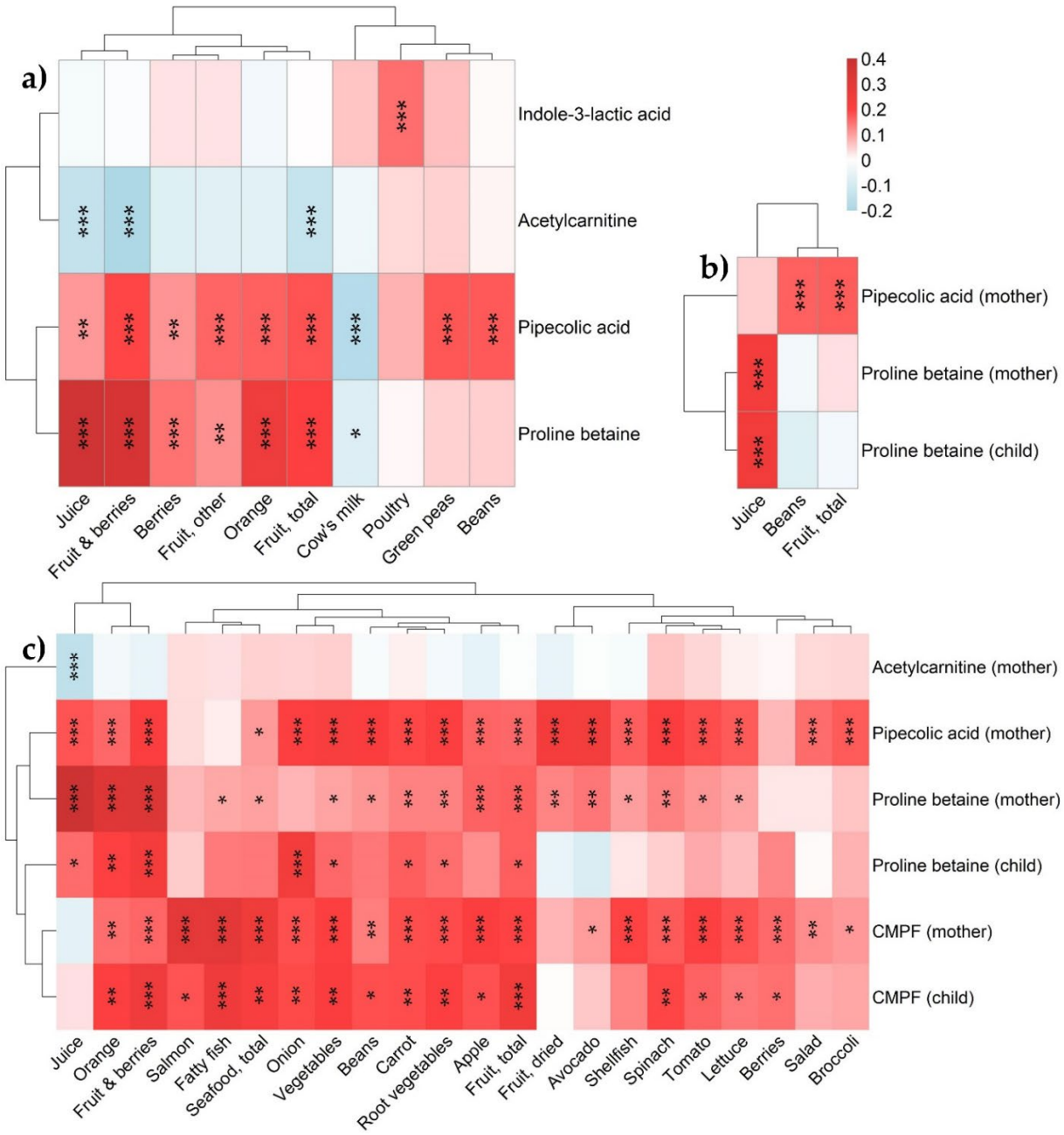

Figure 5. Heatmaps of associations between relative intensities of food intake biomarkers in plasma from **a)** pregnant women, **b)** women and their child at delivery, **c)** women and their breastfed children, in relation to maternal reported food intake (grams/day) at gestational week 30-34, first month postpartum, and between three and four months postpartum, respectively. All variables with any association with p-values <0.001 in **Paper V** are presented in here.

5.3.2. Seafood intake and trace elements

Seafood is a food group often discussed in the field of allergy prevention. Potential candidate biomarkers for seafood intake were investigated in urine and blood samples collected during pregnancy (**Paper IV**). In addition to the more commonly investigated fish fatty acids (DHA, EPA, and DPA), the micronutrients iodine and selenium, and the food chain contaminants arsenic and mercury were investigated since these are broadly found in seafood.

Concentrations of mercury in erythrocytes correlated relatively strongly with FFQ-reported intake of seafood and was by far the most strongly associated objective measurement regardless of seafood type. The candidates with the strongest potential as seafood biomarkers in this cohort are summarized in **Figure 6**, where associations from **Paper IV** with rho>0.25 and p<0.001 are presented. As can be seen, the fatty

acids EPA and DHA, commonly regarded as good biomarkers of fish intake, are not among the biomarkers with the strongest associations to intake of total seafood (rho=0.20 and rho=0.22, respectively), or fatty fish (rho=0.24 and rho=0.25, respectively) during pregnancy. It is possible that the associations would be different during another period of life or in another geographical area. However, a Norwegian study of pregnant women showed similar results with stronger associations between seafood intake and mercury and arsenic levels in blood than with the more commonly used n-3 LCPUFAs [89]. Also, a randomized controlled trial has previously indicated that DHA and EPA (serum phospholipid concentrations) might be good seafood biomarkers during the first trimester, but not from second trimester and onwards [57].

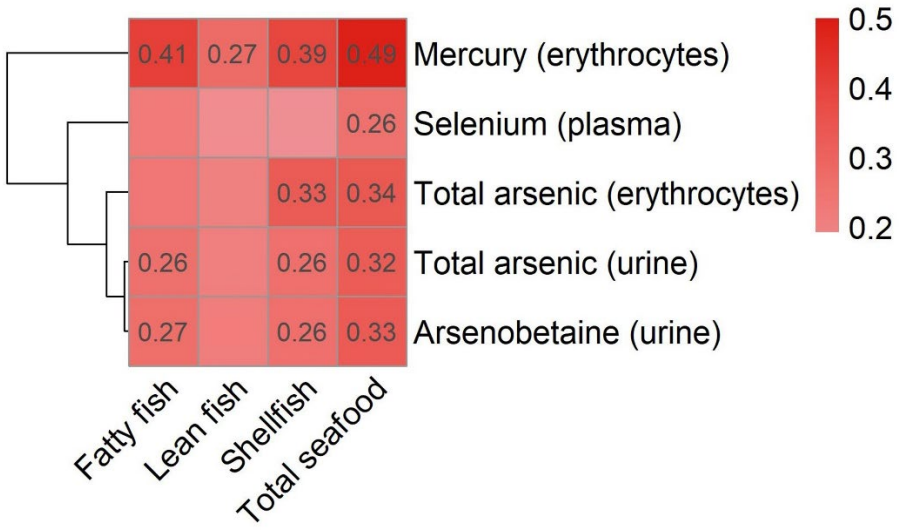

Figure 6. Blood and urine concentrations of candidate biomarkers of seafood intake during pregnancy. Associations with a correlation coefficient above 0.25 and a p-value below 0.001 are summarized. Full results are presented in **Paper IV**.

5.4. Biomarkers to evaluate associations between food intake and allergy

Several associations were identified between self-reported food intake and diagnosis of allergy during the first year of life (**Paper II**). In the thesis, identified metabolites from **Paper V** were used to further investigate the findings from **Paper II**. When it comes to using food intake biomarkers during pregnancy it is important to consider the remarkable physiological changes during pregnancy and the transport of nutrients to the growing fetus. These factors might affect the plasma levels of some candidate biomarkers, making them less reliable as food intake biomarkers during pregnancy.

5.4.1. Ruminant fatty acids, cow's milk intake and food allergy

Several previous studies have used the saturated fatty acids (SFA) pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) as biomarkers of cow's milk intake, as thoroughly reviewed elsewhere [47] and discussed in the background section. The use of these fatty acids as biomarkers of cow's milk intake is often motivated by the fact that they are produced by microorganisms in the rumen of the cows [90]. As presented in **Paper II**, proportions of pentadecanoic acid (15:0) in breast milk correlated with both maternal cow's milk intake at four months postpartum (rho=0.25, $p<0.001$) and directly with offspring food allergy (rho=-0.151, p=0.02), while no such association could be seen for heptadecanoic acid (17:0) and food allergy. To investigate these findings further, a logistic regression with the fatty acid proportion divided by the interquartile range was done, and the results are presented in **Figure 7**. As can be seen, the correlation between higher proportions of C15:0 and lower incidence of food allergy is supported by the findings from the logistic regression (crude and adjusted for heredity: OR=0.43, p=0.01). However, as seen in the figure, these fatty acid proportions (C15:0 and C17:0) at one month postpartum were not associated with lower odds of food allergy. Previous literature regarding maternal cow's milk intake, specifically during lactation, and the risk of offspring food allergy are lacking, as concluded elsewhere [22]. Hence,

future studies are needed to elucidate if there might be a causal relationship between the intake of cow's milk, specifically during lactation, and offspring allergy risk, and if so, at what doses.

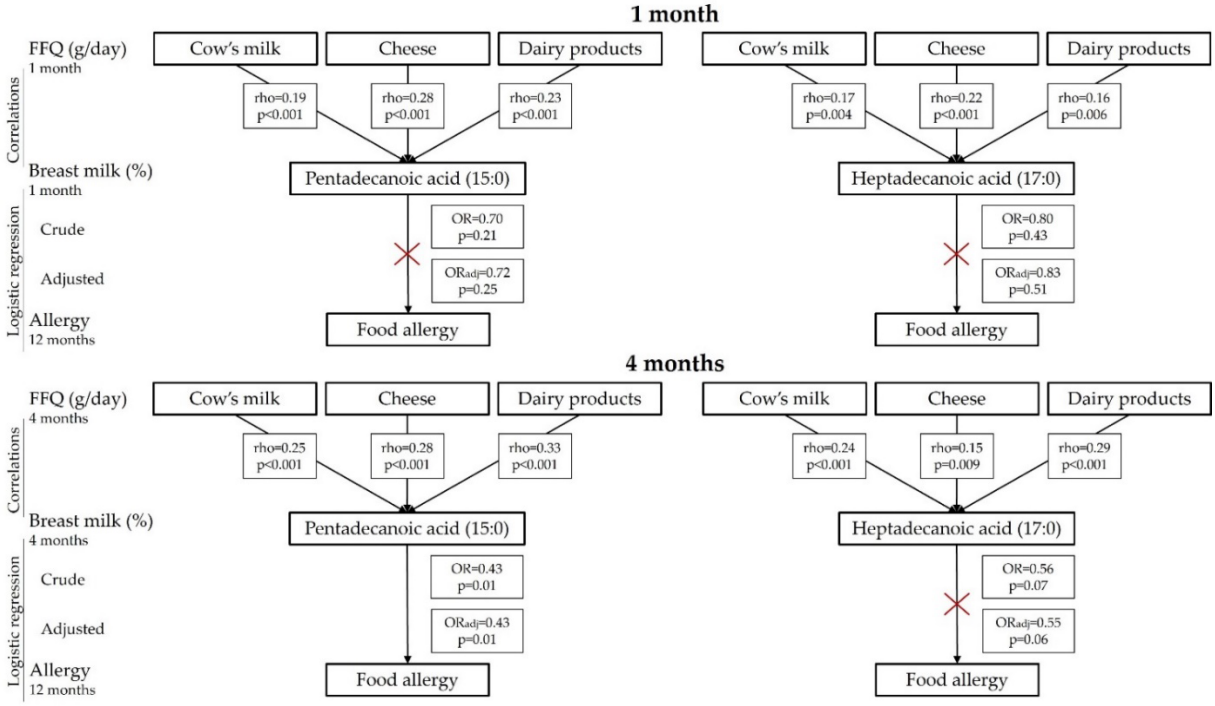

Figure 7. Cow's milk fatty acids in relation to food allergy.
This is a summary of the results found in **Paper II** with additional logistic regression analyses. The odds ratio (OR) is presented per interquartile range (IQR): pentadecanoic acid, 1 month=0.16 and 4 months=0.18; heptadecanoic acid, 1 month=0.09 and 4 months=0.10. The adjusted model included any allergic disease within the family as a covariate. For the statistically significant analysis (C15:0 at four months in relation to food allergy) the crude and adjusted OR (95% CI) were 0.43 (0.23-0.82) and 0.43 (0.23-0.81), respectively.

5.4.2. Proline betaine, fruit and berries intake and eczema

Interestingly, intake of fruit and berries while breastfeeding was associated with a higher incidence of offspring atopic eczema in **Paper II**. As shown in **Paper V**, the relative intensity of proline betaine (a citrus fruit biomarker) in plasma was strongly associated with fruit intake. If the finding in **Paper II** would be of causal origin and explained by the intake of citrus fruit in particular, one could expect the food intake biomarker proline betaine also to be associated with atopic eczema. However, neither maternal nor infant plasma levels at four months were associated with atopic eczema (**Figure 8**). Hence, proline betaine in plasma could not confirm the findings between fruit intake and offspring eczema.

Proline betaine is validated as a food intake biomarker for citrus fruit [91]. In this cohort of pregnant women, proline betaine was most strongly associated with the reported intake of fruit juice. Fruit and berries are, however, a broad group and include food items which probably is not well reflected by proline betaine. A previous study showed the opposite results, where higher maternal fruit intake during pregnancy resulted in lower odds of eczema at three years of age [30]. In that study, stachydrine was used as a biomarker of fruit intake, which seems to be a synonym for proline betaine.

As discussed in **Paper II**, when studying the different types of fruit included in the overall group in relation to atopic eczema, intake of banana and fruit soup/kissel (e.g., bilberry or rose hip) were the ones positively associated with the diagnosis, and hence, the ones who could explain the association between fruit and berries and eczema. The intake of banana and fruit soup/kissel was not related to plasma levels of proline betaine (rho=0.07, p=0.13, and rho=0.08, p=0.08), which could explain why proline betaine was not confirming the association found between fruit and berries and eczema in our cohort. Hence, it is important not to encourage a reduced intake of fruit and berries for allergy prevention since the results

are conflicting. It is possible that the reported intake of fruit and berries represent a type of lifestyle that is associated with eczema and not a causal relationship between fruit intake and eczema.

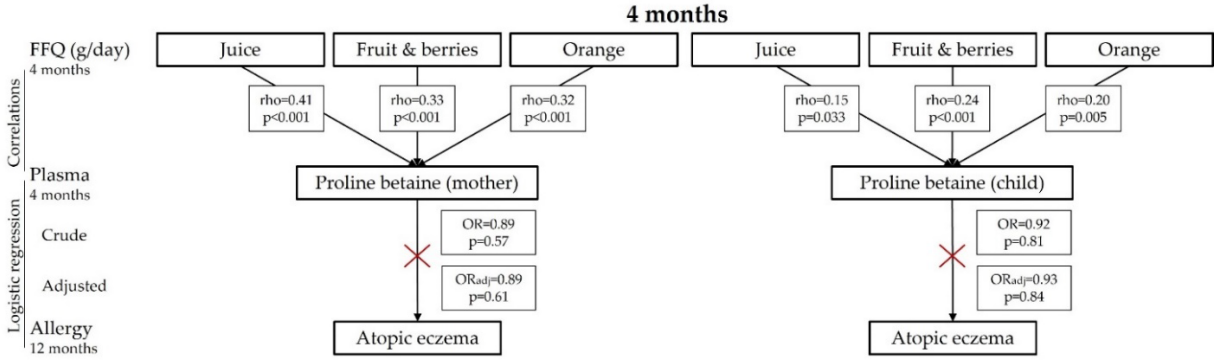

Figure 8. Proline betaine in relation to atopic eczema.

This is a summary of the results found in **Paper V** with additional logistic regression analyses. The odds ratio is presented per interquartile range (IQR). The adjusted model included any allergic disease within the family as a covariate.

5.4.3. Seafood intake, seafood biomarkers, and eczema

In **Paper III**, higher phospholipid proportions of n-6 PUFAs in the infants' umbilical cord at birth were associated with a higher incidence of atopic eczema, both in unadjusted models and after taking allergic heredity into account. These proportions were also negatively related to fish intake during pregnancy (fatty fish, rho=-0.17, p=0.02; total fish, rho=-0.16, p=0.03). These results may indicate that a low fish intake during pregnancy may result in a higher incidence of atopic eczema through higher proportions of n-6 PUFAs. However, no associations were found between the self-reported seafood intake and offspring allergy.

As discussed previously, subjective measures of fish intake may not be very accurate, and objective measures may instead be used. In **Paper IV**, mercury concentrations in erythrocytes and arsenobetaine in urine were found to correlate with the reported intake of most seafood types during pregnancy. Hence, if these objective measurements reflect seafood intake during pregnancy better than the dietary assessment, and if seafood intake could prevent allergy, one could expect a negative association between these concentrations and atopic eczema. In logistic regression models conducted for this thesis, no such association could be found (**Figure 9**).

Taken together the results of this thesis and the existing literature, maternal seafood intake during pregnancy does not seem to affect offspring allergy development during the first year of life. Although some studies have reported a lower incidence of offspring allergy [92, 93], the overall evidence seems to, in accordance with the findings in this thesis, indicate that seafood intake during pregnancy might not be relevant in terms of allergy prevention [36, 37].

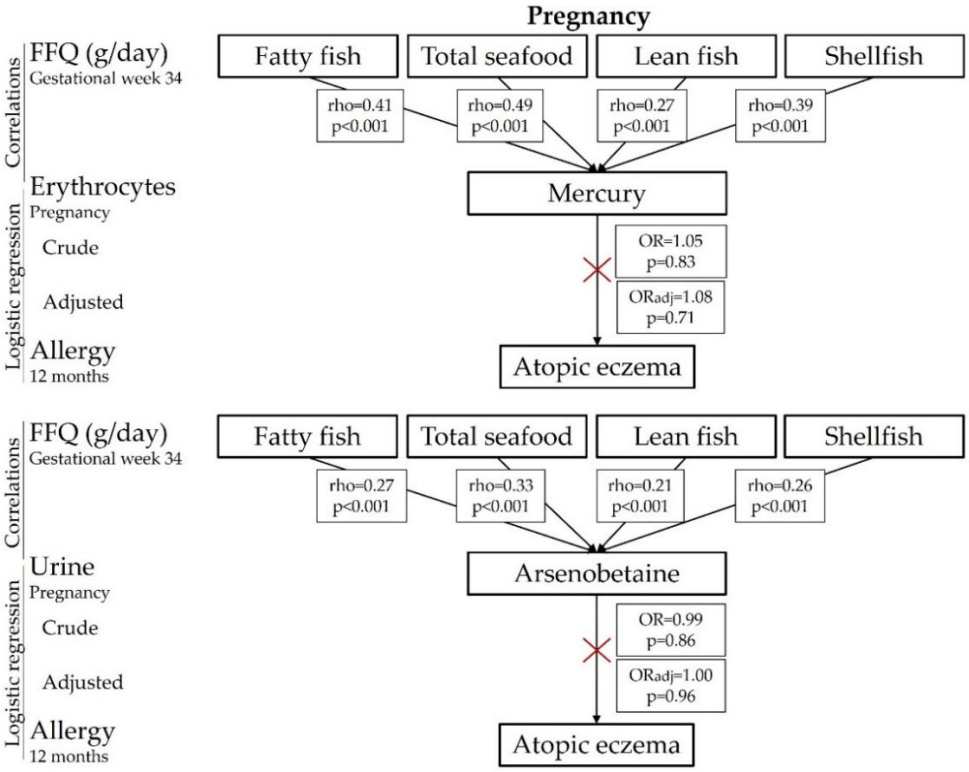

Figure 9. Summary of associations between seafood biomarkers and atopic eczema.

This is a summary of the strongest results found in **Paper IV** (mercury and arsenobetaine). The odds ratio is presented per interquartile range (IQR): mercury=1.35 and arsenobetaine=32.3. The adjusted model included any allergic disease within the family.

6. STRENGTHS AND LIMITATIONS

The work within this thesis has several strengths. For instance, the assessment of maternal diet was extensive, with three repeated semi-quantitative FFQs covering the food intake during pregnancy until four months postpartum. Having information regarding maternal food intake while breastfeeding is quite unique since previous literature focuses primarily on the diet during pregnancy or the postpartum weaning practice. Further, allergy was diagnosed by a pediatrician who specialized in allergy and was, hence, not parent-reported or based solely on sensitization. The same allergologist set the diagnosis throughout the study, meaning that bias potentially introduced by inter-individual judgments has been removed.

Another strength is that the sample collection was extensive. A wide range of biological samples were collected from both the mother and the child at multiple times. The work within this thesis includes data from blood sampling of the mother, the fetus (i.e., umbilical cord), and the infant. Further, it includes repeated breast milk collections and urine samples. Altogether, this enabled the use of metabolomics and measurements of fatty acids and trace elements in different biological samples, in order to investigate the maternal diet more thoroughly and objectively.

However, there are also limitations to acknowledge. For instance, the data curation was not fully finished at the time **Paper I** was published. More specifically, the estimated food intake was therefore based on the reported intake frequency and normal portions as suggested by the Swedish Food Agency (i.e., not portion sizes reported by the study participants). However, the quantification was improved in the following papers by taking reported portion sizes into account. Importantly, no major differences were identified in secondary analyses where the results from **Paper I** were reproduced with the new quantification, and the conclusions remained unchanged.

This thesis focused on studying allergies during children's first year of life, and it is possible that maternal food intake plays a different role in allergies developing further on. Hence, it is important to also investigate the role of maternal diet during pregnancy and lactation on allergies diagnosed later in childhood. In the NICE cohort, all children are currently followed up and assessed for allergy at four and six years of age.

6.1. Causality

The results produced within this thesis are based on observational cohort data, which makes it difficult to draw conclusions regarding the causal effects of maternal diet on offspring allergy risk. Although attempts were made to more objectively rank individuals based on their diet (i.e., food intake biomarkers), the lack of specificity of these biomarkers is a limitation. Further, associations between the food intake biomarkers and self-reported food intake were relatively weak. In causal inference, the strength of association can indeed be seen as a guideline for judging the causality of the findings [94]. However, the lack of strong associations is rather expected in this type of study, and the expected correlation between concentration biomarkers and the actual food intakes has been suggested to be below 0.6 [43]. In addition, since the food intake biomarkers were correlated to self-reported intakes measured with a FFQ which underestimates the actual intakes, the correlation coefficients can, of course, be expected to be even lower. Hence, the lack of strong associations in this thesis does not automatically imply a lack of causality, although it warrants further investigation.

7. CONCLUSIONS

In summary, this thesis presents in-depth information regarding food intake during pregnancy and lactation in the NICE cohort. The development of offspring allergy during the first year of life was associated with maternal intake, primarily during lactation, of cow's milk, saturated fat, and fruit and berries, although causality cannot be proven. Furthermore, infant's fatty acid profile at birth was associated with eczema development during the first year of life. The conclusions of this thesis are:

- Maternal characteristics influence food intake during pregnancy. For instance, higher education and age were associated with a more nutrient-dense diet (including fruit, vegetables, fish, and nuts). Higher BMI in early pregnancy and smoking before pregnancy were related to a lower intake of these food groups.

- Since food intake is closely related to maternal characteristics, it is difficult to determine the specific significance of food intake on different outcomes (e.g., allergy development). The complex interaction between food choice and lifestyle must be considered here as in observational studies in general.

- Maternal intake of cow's milk while breastfeeding, confirmed with the cow's milk intake biomarker C15:0, was associated with a lower incidence of offspring food allergy during the first year of life, even after adjusting for reverse causation. This could indicate that cow's milk might lower the risk of developing food allergy *or* simply that reported intake of cow's milk reflect unmeasured factors which affect allergy risk.

- Maternal intake of saturated fat while breastfeeding was inversely associated with offspring food allergy, eczema, and asthma during the first year of life. This warrants further investigation to elucidate if a higher intake of saturated fat competes with the intake of immunomodulatory PUFAs *or* if saturated fat intake is associated with a certain lifestyle *or* if the saturated fat *per se* might influence allergy development.

- Higher proportions of n-6 PUFAs, foremost arachidonic acid, in umbilical cord plasma phospholipids were associated with a higher incidence of atopic eczema during the first year of life. Lowering the n-6 PUFA proportions *in utero* may be achieved by increasing the proportions of n-3 PUFA. The n-6 and n-3 PUFA proportions in the umbilical cord blood depend on the endogenous production by the mother and child, the transport over the placenta, and the maternal food intake.

- Using food intake biomarkers known from a general (i.e., non-pregnant or lactating) population to reflect the food intake seems applicable also in breastfeeding women. For instance, proline betaine measured in plasma correlated relatively strongly with self-reported intakes of fruit juice, orange, and also a general intake of fruit and berries. The strongest correlation between food intake and exposure biomarkers during pregnancy was noted for mercury in erythrocytes and reported intake of seafood. Further investigations are needed to find metabolomics-based food intake biomarkers during pregnancy.

8. FUTURE PERSPECTIVES

- The causality of the findings in this thesis needs to be investigated. For instance, if it is a higher maternal intake of cow's milk *per se* that protects against food allergies in the offspring or if there are other confounding factors affecting this association. Considering causal inference tools such as the Bradford-Hill criteria [91], there is a need for:
    - Mechanistic studies investigating immunomodulation by separate components of cow's milk, fruit and berries, and fatty acids intake.
    - Randomized controlled intervention studies investigating dose-response for cow's milk, fruit and berries, and fatty acids intake and allergy development.
    - More studies investigating associations between maternal intake of cow's milk, fruit and berries, and fatty acids intake during lactation in relation to offspring allergy development.
- Investigate how objective measurements can be combined with self-reported food intake in order to compensate for the shortcomings of both methods.
- Investigate associations between maternal diet during pregnancy and lactation and offspring allergy development at four and six years of age in the NICE cohort.
- Investigate associations between infant feeding (e.g., timing of food introduction, and diet diversity) and allergy incidence, both alone and combined with maternal dietary exposures.

9. ACKNOWLEDGMENTS

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11. APPENDIX

In order to complement the knowledge summarized in previous reviews, a literature search regarding publications from the last five years was conducted in PubMed using the following search terms:

("Pregnancy"[Title/Abstract] OR "Lactation"[Title/Abstract]) AND ("Diet"[Title/Abstract] OR "Food"[Title/Abstract]) AND ("Allergy"[Title/Abstract] OR "Eczema"[Title/Abstract] OR "Asthma"[Title/Abstract]) AND ("Offspring"[Title/Abstract] OR "Infant"[Title/Abstract] OR "Child"[Title/Abstract]) AND (y_5[Filter]).

The search was conducted in April 2023 and resulted in N=195 hits which were all investigated in terms of title and abstract. From the first round of screening, N=56 made it to the second round. Reasons for exclusion included subject (not concerning maternal diet during pregnancy or lactation in relation to offspring allergy, N=86), language (not available in English, N=3), animal studies (mice, N=4), and reference type (not original research, N=46: guideline, N=2; review, N=33; study protocol, N=6; meta-analysis, N=2; book chapter, N=2; and correction, N=1).

In the second round, the articles were read more thoroughly, and N=21 were thereafter excluded since they did not concern maternal diet during pregnancy or lactation (e.g., investigated supplementation) in relation to offspring food allergy, eczema, or asthma (e.g., investigated wheeze or immunological markers). The remaining publications (N=35) are summarized in **Table A1** with a focus on results concerning food allergy, eczema (atopic dermatitis and atopic eczema were handled interchangeably), and asthma during childhood (i.e., the publications might include results concerning adolescents or other allergy diagnoses which are not summarized in the table).

Table A1. Overview of original research papers published the last five years regarding maternal food intake and offspring food allergy, eczema, or asthma.

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Amberntsson et al. (2022) [1] *GraviD*	Vitamin D *Pregnancy*	FFQ* Reflecting: Late pregnancy (after GW 31) Filled in: Late pregnancy (after GW 31) **Vitamin D Questionnaire (VDQ) assessing intake of fatty fish, milk, yoghurt and/or sour milk, and margarine.*	FA Ecz Asth ≤5 yrs	Parent-reported *Questionnaire* Filled in at 5 years, reflecting allergy at any time during these years	FA: No effect ↑ Ecz = ↑ Vitamin D (diet)* OR (95% CI): 1.14 (1.01-1.29) *Per increase of µg/day* Asth: No effect *Only for children without allergic heredity	606	FA: 111 (18%) Ecz: 133 (22%) Asth: 76 (13%) *Cumulative incidence* 189 (31%) had allergic heredity
Azad et al. (2021) [2] Canada *CHILD*	Peanuts *Pregnancy (Lactation)*	FFQ Reflecting: Pregnancy (lactation*) Filled in: Mid-pregnancy **Used the reported intakes during pregnancy and assumed the intake was similar* Excluded those with regular peanut & nuts intake but without peanut butter intake.	FA* 3 yrs **Probable IgE-mediated peanut allergy*	SPT, symptom history, consumption* *Less than once per month	FA: No effect (alone)* **Only if weekly consumption in combination with breastfeeding & early peanut introduction (<1 yr)* Triple exposure hypothesis	2759	46 (1.9%)
Baïz et al. (2019) [3] *EDEN*	General diet *Pregnancy*	FFQ Reflecting: Late pregnancy (third trimester) Filled in: After delivery (first days)	Ecz Asth ≤3 yrs	Parent-reported diagnosis *Questionnaire (ISAAC)* 1, 2 & 3 yrs	Ecz: No effect Asth: No effect	1140	Asth: 10% Ecz: 43% *Cumulative incidence*
Barman et al. (2021) [4] Sweden *NICE*	General diet *Pregnancy*	FFQ Reflecting: GW 30-34 Filled in: GW 34 Fatty acid proportions measured in plasma phospholipids at birth	Ecz ≤1 yr	Doctor's diagnosis	↑ Ecz = ↑ n-6 PUFAs* **Proportions in cord blood* OR (95% CI): 1.67 (1.11-2.51) *Increase of one IQR* ↓ n-6 PUFAs = ↑ Fatty fish ↑ Total seafood	206	14 (7%) >50% (i.e., eight infants) also had other allergies

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Brustad et al. (2023)[1] [5] Denmark *COPSAC$_{2010}$*	Diet-related metabolites *Pregnancy*	FFQ Reflecting: GW 20-24 Filled in: GW 24 Metabolites measured in dried blood spot (plasma) collected from infant heel 2-3 days after delivery	Ecz Asth* ≤6 yrs **Wheeze at 3 yrs*	Doctor's diagnosis *Respiratory symptoms were prospectively recorded (daily diary)*	<u>3 years</u> ↓ Ecz = ↑ Stachydrine* OR (95% CI): 0.74 (0.58-0.95) ↑ Stachydrine = ↑ Fruit <u>6 years</u> Ecz: No effect ↓ Asth = ↑ Paraxanthine[1]* ↑ Caffeine[2]* [1] OR (95% CI): 0.69 (0.50-0.95) [2] OR (95% CI): 0.67 (0.48-0.94) **Coffee-related metabolites*	653	*Unclear*
Brzozowska et al. (2022) [6] Poland *REPRO_PL*	General diet *Pregnancy*	FFQ Reflecting: Average consumption Filled in: GW 20-24 Intake evaluated by subtracting *Western Dietary Pattern (WDP)* score from *Prudent Dietary Pattern (PDP)* score Micronutrients were compared to EAR	FA Ecz Asth* 1, 2 & 7-9 yrs **Wheeze at 1 & 2 yrs*	Doctor's diagnosis *Questionnaire (ISAAC), medical records, SPT & spirometry*	<u>1 year</u> FA: No effect Ecz: No effect <u>2 years</u> FA: No effect ↑ Ecz = ↓ Vitamin E (diet) OR (95% CI): 2.74 (1.08-7.73) *Meeting EAR as reference* <u>7-9 years</u> FA: No effect ↓ Ecz = ↑ Prudent diet* **Calculated as PDP-WDP* OR (95% CI): 0.66 (0.47-0.93) Asth: No effect	557	<u>1 year</u> FA: 59 (17%) Ecz: 76 (22%) <u>2 years</u> FA: 37 (17%) Ecz: 39 (18%) <u>7-9 years</u> FA: 66 (20%) Ecz: 67 (20%) Asth: 52 (15%)

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Chen et al. (2020) [7] Ireland *Lifeways Cross-Generation*	Inflammatory diet *Pregnancy*	FFQ Reflecting: Habitual intake Filled in: First trimester Dietary Inflammatory Index (E-DII) and Healthy Eating Index (HEI)	Asth 3, 5, & 9 yrs	Parent-reported and doctor's diagnosis* **Reported by general practitioners at 3 and 9 yrs, and by parents at 5 and 9 yrs*	<u>3 years</u> ↑ Asth = ↑ E-DII[1] ↓ Asth = ↑ HEI[2] [1]OR (95% CI): 1.44 (1.06, 1.95) [2]OR (95% CI): 0.70 (0.53, 0.93) <u>5 years</u> ↑ Asth = ↑ E-DII OR (95% CI): 1.42 (1.04, 1.94) <u>9 years</u> ↑ Asth = ↑ E-DII OR (95% CI): 1.36 (1.03, 1.79) <u>First 10 years</u> ↑ Asth = ↑ E-DII[1] ↓ Asth = ↑ HEI[2] [1]OR (95% CI): 1.35 (1.10, 1.65) [2]OR (95% CI): 0.77 (0.64, 0.93) *Per increment of 1 SD*	862	3 yrs: 10% 5 yrs: 13% 9 yrs: 23% *Cumulative incidence*
Chen et al. (2022) [8] China *TMCHC*	Low-carbohydrate diet *Pregnancy*	FFQ Reflecting: Last month Filled in: GW 35.6 ± 2.3 Low-carbohydrate diet (LCD) score based on E% from each macronutrient. ↑ LCD score = ↓ E% from carbohydrates *Women with a higher LCD score had higher education, age, BMI, and more often had gestational diabetes*	FA Ecz* Asth*] "IgE" ≤2 yrs **Merged with allergic rhinitis as IgE-mediated allergies*	Parent-reported diagnosis *Interviews over phone & questionnaire*	↑ FA = ↑ Animal protein/fat* RR (95%CI): 1.28 (1.00-1.63) **Substitution of carbohydrates with animal protein and fat. Middle quintile of LCD score as reference.* ↓ "IgE" = ↑ Protein (15E%) *RR (95%CI): 0.60 (0.41-0.87) *10E% as reference* ↑ "IgE" = ↑ Animal fat (22E%)[1] ↑ LCD score (highest)[2] ↓ LCD score (lowest)[3] [1]RR (95%CI): 1.52 (1.03-2.22) *14E% as reference* [2]RR (95%CI): 1.72 (1.22-2.63) [3]RR (95%CI): 1.77 (1.13-2.77) *Middle quintile of LCD score as reference*	1636	FA: 488 (30%) "IgE": 230 (14%)

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Delvert et al. (2022) [9] France *EDEN*	General diet *Pregnancy*	FFQ Reflecting: Last trimester Filled in: After delivery (unclear time) Diet Quality Score (food intake) and PANDiet (nutrients) for adherence to France dietary guidelines	FA* Ecz* Asth* ≤8 yrs **Represented in several clusters*	Parent-reported diagnosis *Questionnaire (ISAAC)* Clusters: 1) Asymptomatic, 2) Asth only, 3) Allergy, and 4) Multiallergic *The different clusters include all allergies (i.e., FA is present also in "Asth only" group)*	↑ Multiallergic = ↓ Legumes *OR (95%CI): 1.60 (1.01-2.54) *Asymptomatic cluster and >1 per month as reference (vs ≤1 per month)*	1593	FA: 181 (11%) Ecz: 414 (26%) Asth: 295 (19%) *Cumulative incidence*
Flom et al. (2021) [10] US *PRISM*	PUFA (n-3 & n-6) *Pregnancy*	FFQ Reflecting: Last three months Filled in: Second trimester *Intake both from diet and supplementation*	Asth ≤4 yrs	Parent-reported *Interviews*	↑ Asth = ↓ n-3 PUFA intake (↑) n-6 PUFA intake* **Mid-range indicated lowest risk (i.e., lower intake is not necessarily better)* Strongest if mother had asthma Strongest effect among girls	412	72 (17%) Median debut=3.5 yrs *22% of the mothers had asthma*
Gao et al. (2019) [11] China *No name*	General diet *Pregnancy*	FFQ Reflecting: Whole pregnancy Filled in: After delivery (unclear when) Milk, egg, beans, nuts, aquatic products* **Fish, shrimp, crab, and shellfish*	FA Ecz ≤1 yr	Parent-reported diagnosis *or* symptoms *Questionnaires*	↑ FA = ↑ Aquatic products *OR (95% CI)=1.73 (1.12-2.67) *1-2 times/week vs <1 times/week* ↓ FA = ↑ Egg *OR (95% CI)=0.61 (0.38-0.99) *3-4 times/week vs ≤2 times/week* ↓ Ecz = ↑ Egg *OR (95% CI)=0.51 (0.32-0.81) *3-4 times/week vs ≤2 times/week* ↑ Ecz = ↑ Milk *OR (95% CI)=1.81 (1.17-2.80) *3-4 times/week vs ≤2 times/week*	903	FA: 200 (22%) Ecz: 226 (25%)

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Hanson et al. (2020) [12] *Project Viva*	Inflammatory diet *Pregnancy*	FFQ x2 Reflecting: First two trimesters* Filled in: GW 10 & GW 30 **Intake since last menstrual period (filled in GW10) & last three months (filled in GW30)* Dietary Inflammatory Index (DII)	Asth ≤9 yrs* **Median: 3.3 & 7.7*	Parent-reported *ISAAC*	Asth: No effect	1424	3 yrs: 11% 8 yrs: 21% *Ever diagnosed*
Kim et al. (2019) [13] Korea *COCOA*	General diet *Pregnancy*	FFQ Reflecting: Habitual intake (last year) Filled in: GW 26 5 dietary patterns: 1) Confectionary (e.g., bread, potato, sweets), 2) Traditional (e.g., vegetables, seaweed, fruits, kimchi), 3) Meat (e.g., fish, pork, chicken), 4) Processed (e.g., fast food, fat, cheese), and 5) Coffee and milk	FA 1 yr	Doctor's diagnosis	↑ FA = ↑ Confectionary diet *OR (95% CI): 1.52 (1.02-2.15) *Reference low pattern score (first and second vs third and fourth quartile)* ↑ Confectionary diet = ↑ Trans fat	1628	147 (9%)
Kuśmierek et al. (2019) [14] *No name*	General diet *Pregnancy*	Questionnaire Reflecting: Third trimester Filled in: Retrospectively* **Not specified at what time.*	FA (CMA) Unclear age* **Inclusion between 3-24 weeks of age and a criteria was CMA*	Doctor's diagnosis *Oral food challenge*	↓ FA = fish intake 2-3 times/week *OR (95%)=0.16 (0.05-0.61) *Reference intake unclear* 48% (control) vs 18% (CMA) with fish intake 2-3 times/week	76	51 *Case-control (i.e., prevalence not applicable)*
Lamminsalo et al. (2022) [15] *DIPP*	Fatty acids *Pregnancy*	FFQ Reflecting: Gestational month 8 Filled in: After delivery* **Mailed after delivery and returned three months postpartum*	FA (CMA) 3 yrs	Registry for reimbursement (infant formula) & questionnaire *Diagnosis often with oral food challenge*	FA: No effect* *Interaction of maternal heredity on intake of ALA and offspring CMA: OR (95% CI): No: 0.72 (0.56, 0.93) Yes: 1.05 (0.88, 1.26)	4921	448 (9%)

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Melero et al. (2020)[1] [16] Spain *St. Carlos GDM prevention study*	Mediterranean diet* *Pregnancy* **Intervention group encouraged to eat extra virgin olive oil and pistachios* *Control group asked to restrict intake of fat (e.g., olive oil and pistachios)*	FFQ (for adherence) *Mediterranean diet score (MEDAS)* Reflecting: Intake from GW 12 Filled in: GW 12, 24 & 36 All were instructed to follow a general Mediterranean diet Intervention group met with dietitian weekly and were given extra virgin olive oil and pistachios to consume daily (40 mL and 25-30 g)	FA Ecz Asth 2 yrs	Medical records, prescriptions, and interview *Small for gestational age more common in control group (6.3% vs 1.5%, p=0.002)*	No effect	703	<u>All</u> FA: 50 (7%) Asth: 18 (3%) Ecz: 207 (29%) <u>Control</u> FA: 29 (8%) Asth: 7 (2%) Ecz: 101 (28%) <u>Intervention</u> FA: 21 (6%) Asth: 11 (3%) Ecz: 106 (32%)
Milewska-Wróbel et al. (2022) [17] *No name*	Antioxidants & n-3 PUFA *Pregnancy*	Questionnaire Reflecting: Whole pregnancy Filled in: After delivery (unclear time) Intake of antioxidative and n-3 FA rich food Six categories: 1) fruits and vegetables, 2) chocolate confectionery, 3) whole grain breakfast cereals, 4) nuts and seeds, 5) coffee, tea, fruit juices, 6) fish and fish products	FA Ecz Asth ≤3 yrs	Doctor's diagnosis *Medical records* All were diagnosed with Ecz before enrollment	FA: No effect Ecz: No effect (debut, severity) ↑Asth = ↓ Fruit and vegetables[1] ↓ Chocolate[2] [1]OR (95% CI): 0.05 (0.00, 0.92) [2]OR (95% CI): 0.10 (0.01, 0.95) *Reference unclear* *Large variation in intakes in the group without Asth*	100	FA: 33 (33%) Ecz: 100 (100%) Asth: 9 (9%) *Ecz debut most common between 3-6 months*
Nakano et al. (2023) [18] Japan *JECS*	Mediterranean diet *Pregnancy*	FFQ Reflecting: Mid-late pregnancy Filled in: Second-third trimester Three scoring systems for adherence to Mediterranean diet: MDS, rMED, and PMDS (dairy are scored positively and system adjusted for pregnancy)	FA Ecz Asth 4 yrs	Parent-reported *Questionnaire* Excluded children born with cesarean section	FA: No effect Ecz: No effect ↓ Asth = ↑ PMDS* **Prevalence lower with low vs high score (8% and 9%). No multivariate statistics (i.e., not adjusted for covariates)*	46 532	Any: 11 293 (24%) FA: 2616 (6%) Ecz: 3954 (8%) Asth: 3815 (8%) *50% of the mothers had allergy*

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Ogawa et al. (2021) [19] Japan *JECS*	Vegetables *Pregnancy*	FFQ Reflecting: Pregnancy (unclear time) Filled in: GW 22-28 Categorized vegetables into 3 groups 1) Folate-rich (100 µg/100 g): e.g., spinach, broccoli, spring onion, 2) Green and yellow (600 µg carotene/100g): e.g., carrot, pumpkin, tomato, 3) Cruciferous: "mustard family" (e.g., cabbage, radish) *Some vegetables included in all three groups (e.g., broccoli, Japanese mustard spinach)*	FA Ecz Asth 1 yr	Parent-reported diagnosis *Questionnaire (ISAAC)*	↑ FA = ↑ Total vegetables[1] ↑ Green & yellow[2] *[1]95, 161 & 264 vs 56 g/day* *[2]43, 58 & 102 vs 16 g/day* ↑ Ecz = ↑ Total vegetables[1] ↑ Folate-rich[2] ↑ Green & yellow[3] *[1]161 & 264 vs 56 g/day* *[2]69 vs 9 g/day* *[3]58 & 102 vs 16 g/day* ↓ Asth = ↑ Cruciferous[1] *[1]21 & 75 vs 10 g/day* All OR were close to one, indicating no (or very small) impact of maternal vegetable intake on any of the allergies	80 270	Any: 25 028 (31%) FA: 13 801 (17%) Ecz: 18 406 (23%) Asth: 2027 (2.5%)
Parr et al. (2018) [20] Norway *MoBa*	Vitamin A & D *Pregnancy*	FFQ Reflecting: Pregnancy (from start) Filled in: GW 20 Sandwich meats (e.g., liver spread), margarine (fortified), and dairy products contributed the most to vitamin A Higher intakes with older age, higher education, primiparity, lower BMI, less smoking, and supplement use	Asth 7 yrs	Prescription database	↑ Asth = ↑ Vitamin A (2031 RAE*) RR=1.21 (1.05, 1.40) *Reference was 779 RAE/day* **>2.5 of RI* ↓ Asth = ↑ Vitamin D (13.5 µg) RR=0.81 (0.67, 0.97) *Reference was ≤3.5 µg/day*	61 676	2546 (4%)
Pretorius et al. (2019) [21] *No name*	Fiber *Pregnancy*	FFQ Reflecting: GW 32-36 Filled in: GW 36-40	FA Ecz ≤1 yr	Doctor's diagnosis, SPT, and parent-reported *All had allergic heredity*	FA: No effect ↑ Ecz = ↑ Resistant starch OR (95% CI): 1.19 (1.01-1.41) *Reference unclear but variable measured in g/day** **Median (IQR): 2.8 (2.1–3.5) g/day*	639	FA: 91 (14%) Ecz: 215 (34%)

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Shimizu et al. (2022) [22] Japan *JECS*	Vitamin D *Pregnancy*	FFQ Reflecting: Mid-late pregnancy Filled in: Mid-late pregnancy Vitamin D intake (quintiles): 1.0, 2.6, 3.9, 5.5, and 10.6 µg/day	FA Ecz Asth 1 yr	Parent-reported diagnosis *Questionnaire*	↑ FA = ↑ Vitamin D (3.9[1] & 5.5 µg[2]) OR (95% CI): 1.13 (1.02-1.25) OR (95% CI): 1.12 (1.01-1.24) *Reference 1.0 µg/day* Ecz: No effect ↑ Asth = ↑ Vitamin D (2.6 µg) OR (95% CI): 1.22 (1.04-1.43) *Reference 1.0 µg/day* No associations found for highest intake (10.6 µg/day)	82 592	FA: 5458 (7%) Ecz: 3551 (4%) Asth: 2048 (2%)
Stråvik et al. (2020) [23] Sweden *NICE*	General diet *Pregnancy* *Lactation*	FFQ x3 Reflecting: Last month Filled in: GW 34, 1 month, 4 months	FA Ecz Asth ≤1 yr	Doctor's diagnosis	<u>Pregnancy</u> ↑ FA = ↑ Poultry ↓ FA = ↑ Cheese Ecz: No effect ↓ Asth = ↑ Game meat <u>Lactation, 1 month</u> ↓ FA = ↑ Cow's milk Ecz: No effect ↓ Asth = ↑ Red meat ↑ Vegetarian dishes <u>Lactation, 4 months</u> ↓ FA = ↑ Cow's milk ↑ Dairy products ↑ Pizza ↑ Ecz = ↑ Fruit & berries ↑ Nuts & seeds ↓ Asth = ↑ Game meat ↑ Processed meat	508	FA: 39 (8%) Ecz: 33 (6%) Asth: 33 (6%)
Stråvik et al. (2021) [24] Sweden *NICE*	Iodine & Selenium *Pregnancy* *Lactation*	FFQ x2 Reflecting: One month back Filled in: GW 34 and 4 months	FA Ecz Asth ≤1 yr	Doctor's diagnosis	<u>Pregnancy</u> No effect <u>Lactation</u> FA: No effect Ecz: No effect ↓ Asth = ↑ Iodine (diet) OR (95% CI): 0.90 (0.82-0.97) *For an increase of 10 µg/day*	588	FA: 41 (7%) Ecz: 35 (6%) Asth: 34 (6%)

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Tan et al. (2023) [25] China *TMCHC*	Yoghurt *Pregnancy* Reflecting: Last month Filled in: Late pregnancy* **After GW 28 (average GW 35)* Consumption group ("any" yoghurt) had vaginal birth more often (60% vs 54%)	FFQ Reflecting: Last month Filled in: Late pregnancy* **After GW 28 (average GW 35)* Consumption group ("any" yoghurt) had vaginal birth more often (60% vs 54%)	Ecz 3 & 6 m	Parent-reported diagnosis	<u>0-3 months</u> Ecz: No effect <u>0-3 and 3-6 months*</u> ↓ Ecz = ↑ Yoghurt (>50 g) RR (95% CI): 0.36 (0.15-0.85) *Reference was no intake* **Recurrent eczema diagnosed at both time points*	3 m: 2371 6 m: 2114	3 m: 182 (8%) 6 m: 84 (4%) *Point prevalence* ≤6 m: 221 (10%) 3 *and* 6 m: 29 (1%)
Tanaka et al. (2021) [26] Japan *KOMCHS*	Caffeine *Pregnancy* Reflecting: Previous month Filled in: GW 5-39 Assessed intake of tea, coffee, soft drink, hot chocolate, and confectionaries Contribution to caffeine: 79% tea, 13% coffee, 4% confectionaries, and 4% soft drinks	Diet history questionnaire Reflecting: Previous month Filled in: GW 5-39 Assessed intake of tea, coffee, soft drink, hot chocolate, and confectionaries Contribution to caffeine: 79% tea, 13% coffee, 4% confectionaries, and 4% soft drinks	FA ≤3 yrs	Parent-reported diagnosis *or* acute reaction *Questionnaires*	↑ FA = ↑ Caffeine (232 mg)* HR (95% CI): 1.46 (1.10-1.96) *Reference 105 mg/day* **Highest intake (410 mg/day) was not associated with FA*	1522	≤3 yrs: 19% 1 yr: 107[1] (7.0%) 2 yrs: 131[1] (8.6%) 3 yrs: 105[1] (6.9%) [1]*Only % presented (i.e., estimated from total sample size)*
Tuokkola et al. (2021) [27] Finland *DIPP*	Antioxidants *Pregnancy* Reflecting: Gestational month 8 Filled in: After delivery	FFQ Reflecting: Gestational month 8 Filled in: After delivery	FA (CMA) ≤3 yrs	Medical records All infants had genetic risk of type 1 diabetes (14% of all infants in Finland)	↑ FA = ↑ β-carotene (diet) OR (95% CI): 1.10 (1.01, 1.19) *Per increment of 1 SD* <u>Sub analysis*</u> ↓ FA = ↑ Selenium (diet) OR (95% CI): 0.85 (0.74, 0.98) *Per increment of 1 SD* ↑ FA = ↑ Zinc (total)[1] ↑ β-carotene (total)[2] [1] OR (95% CI): 1.13 (1.00-1.27) [2] OR (95% CI): 1.12 (1.00-1.26) *Per increment of 1 SD* ** Included parental heredity and pets as additional covariates (N=2327)*	4403	409 (9.3%)

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Venter et al. (2021) [28] *Healthy Start study*	Advanced glycation end products (AGEs) *Pregnancy*	24h recalls Reflecting: Last 24h Filled in: Monthly* **From second trimester*	FA Ecz Asth ≤8 yrs	Medical records	No effect	962	≤1 yr FA: 9 (1%) Ecz: 118 (12%) Asth: 48 (5%)
Venter et al. (2021) [29] US *Healthy Start study*	Inflammatory diet *Pregnancy*	24h recalls Reflecting: Last 24h Filled in: Monthly* **From second trimester* DII scores intakes of inflammatory food (e.g., trans fat) but also anti-inflammatory food which get negative scores (e.g., n-3 PUFAs) *Did not include β-carotene as anti-inflammatory*	Asth* ≤4 yrs **and/or wheeze*	Medical records	Asth: No effect	1228	Unclear* **Same cohort has presented below*
Venter et al. (2022) [30] US *Healthy Start study*	Allergy preventive diet *Pregnancy*	FPQ x2* Reflecting: Last three months Filled in: GW 17 & GW 27 *Developed a new index (0-100) to measure maternal diet in relation to offspring allergy, with higher scores indicating better allergy prevention Scored intake of vegetables, yoghurt, fried potato, rice and grains, red meat, fruit juice (pure), and cereals (cold) since these were associated with any allergy Score ≥ Median = Preventive	FA Ecz Asth ≤4 yrs	Medical records	FA: No effect ↓ Ecz = ↑ FPQ score OR (95% CI): 0.77 (0.69-0.86) *Per 1 unit (score)* ↓ Asth = ↑ FPQ score OR (95% CI): 0.84 (0.74-0.96) *Per 1 unit (score)* Vegetables and yoghurt lowered the odds, while the other five groups increased the odds	1253	Any: 33% FA: 40 (3%) Ecz: 327 (26%) Asth: 182 (15%) *Cumulative incidence*

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Venter et al. (2022) [31] US *Healthy Start study*	Allergy preventive diet *Pregnancy*	FPQ* Reflecting: Last three months Filled in: Mid-pregnancy & delivery **See detailed information above.*	Ecz Asth ≤8 yrs	Medical records <u>Three groups:</u> 1) children with FLG* mutation 2) children without FLG mutation + no maternal allergy preventive diet 3) children without FLG mutation + maternal allergy preventive diet **Filaggrin loss-of-function mutations*	↑ Ecz = Group 2 HR (95% CI): 1.85 (1.23-2.78) *Reference group 3* ↑ Asth = Group 2 HR (95% CI): 1.80 (1.07-3.02) *Reference group 3* FLG mutation did not modify the effect of maternal diet on allergy. The risk of allergy was similar between FLG mutation (group 1) and not eating a preventive diet (group 2).	624	Unclear* **Figures with rates* FLG mutation (i.e., not allergy prevalence): 49 (8%)
Venter et al. (2023) [32] US *Healthy Start study*	General diet *Pregnancy*	FPQ Reflecting: Last three months Filled in: Mid-pregnancy & delivery Maternal diet index (MDI), Healthy Eating Index (HEI), and diet diversity (total, healthy, and unhealthy)	FA Ecz Asth ≤4 yrs	Medical records	FA: No effect ↓ Any = ↑ MDI[1] ↑ HEI[2] ↑ Healthy diet diversity[3] [1]OR (95% CI): 0.78 (0.70-0.87) [2]OR (95% CI): 0.98 (0.97-0.99) [3]OR (95% CI): 0.91 (0.85-0.98) Similar results for Ecz and Asth	1218	Any: 397 (33%) FA: 40 (3%) Ecz: 314 (26%) Asth: 174 (14%)
Zeng et al. (2021) [33] China *No name*	Protein *Pregnancy*	FFQ Reflecting: GW 16-24 Filled in: GW 20-28 Assessed seafood, red meat, poultry, egg, vegetables, fruit, dairy, nuts, cereals, soybean <u>Four patterns</u>: 1) poultry, 2) plant, 3) dairy and egg, and 4) red meat and fish	Ecz ≤6 m	Questionnaire *Interview over phone*	↑ Ecz = ↑ Poultry ↓ Ecz = Plant pattern[1] Dairy and egg pattern[2] [1]OR (95% CI): 0.57 (0.33-0.99) [2]OR (95% CI): 0.48 (0.27-0.84) *Reference Poultry pattern*	713	365 (51%)

	Diet & time	Dietary assessment	Allergy & age	Outcome assessment	Findings	N	N allergy
Zulyniak et al. (2020) [34] Canada *The NutriGen Alliance*	General diet *Pregnancy*	FFQ Reflecting: Pregnancy* Filled in: GW 24-28 **Time not specified (several cohorts)* <u>Three patterns</u>: 1) Plant-based: fermented dairy, legumes, vegetables, whole grains (avoided meat), 2) Western: meat, sweets, fat, pasta, and 3) Balanced: Meat (seafood, poultry, meat dishes), vegetables, fruit	Ecz 1 yr	Parent-reported diagnosis	↓ Ecz = ↑ Plant-based pattern[1] ↑ Western pattern[2] [1]OR (95% CI): 0.65 (0.55, 0.76) [2]OR (95% CI): 0.73 (0.60-0.89) *Per unit increase in PCA score*	2160	613 (28%)
Øien et al. (2019)[1] [35] *PACT*	Fish *Pregnancy* *Lactation*	FFQ x3 Intervention encouraged increased intake of oily fish (2 times/week) and cod liver oil (5 mL/day), reduce smoking and indoor dampness	Ecz Asth 6 yrs	Parent-reported *Questionnaire (ISAAC)*	No effect	4264	*Unclear*

All presented estimates are for confounder-adjusted results. Differences in presentation are due to differences in method descriptions in the original articles.
[1] Based on data from intervention trial (if not indicated with number, data was purely observational).

Abbreviations: Asth, asthma; CMA, cow's milk protein allergy; EAR, estimated average requirement; Ecz, eczema; FA, food allergy; FFQ, food frequency questionnaire; FPQ, Food propensity questionnaire; GW, gestational week; HR, hazard ratio; OR, odds ratio; RAE, retinol activity equivalents; RR, relative risk/risk ratio; yrs, years.

Studies: CHILD, Canadian Healthy Infant Longitudinal Development; COCOA, COhort for Childhood Origin of Asthma and allergic diseases; DIPP, Finnish Type 1 Diabetes Prediction and Prevention study; ISAAC, International Study of Asthma and Allergies in Childhood; JECS, Japan Environment and Children's Study; KOMCHS, Kyushu Okinawa Maternal and Child Health Study; MoBa, Norwegian Mother and Child Cohort Study; NICE, Nutritional impact on Immunological maturation during Childhood in relation to the Environment study; PACT, Prevention of Allergy among Children in Trondheim; PRISM, PRogramming of Intergenerational Stress Mechanisms; REPRO_PL, Polish Mother and Child Cohort; TMCHC, Tongji Maternal and Child Health Cohort;

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