MATERNAL POLYUNSATURATED FATTY ACID STATUS AND OFFSPRING ALLERGIC DISEASES UP TO THE AGE OF 18 MONTHS

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in this thesis.

This thesis has also not been submitted for any degree in any university previously.

YU Ya-Mei

12 May 2014

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SUMMARY

Studies have suggested that maternal polyunsaturated fatty acid (PUFA) status during pregnancy may influence early childhood allergic diseases, although findings are inconsistent. We examined the relation between maternal PUFA status and risk of allergic diseases in early childhood in an Asian study. Maternal plasma samples (n=998) from the GUSTO mother-offspring cohort were assayed at 26-28 weeks of gestation for relative abundance of PUFAs. Offspring were followed up from 3 weeks to 18 months of age, and clinical outcomes of potential allergic diseases (rhinitis, eczema, and wheezing) were assessed by repeated questionnaires. Skin prick testing (SPT) was also performed at age 18 months. An allergic disease was defined as having any one of the clinical outcomes plus a positive SPT. The prevalences of a positive SPT, rhinitis, eczema, wheezing and any allergic disease were 14.1% (103/728), 26.5% (214/808), 17.6% (147/833), 10.9% (94/859), and 9.4% (62/657) respectively.PUFAs of interest were first independently analyzed as continuous variables to test for linear associations with various allergic outcomesi.e. SPT, rhinitis, eczema, wheezing and any allergic disease with positive SPT in the offspring using multiple linear regression models. To test for a possible non-linear relationship and to examine dose-response, the PUFAs were next categorized into quartiles within the total cohort, and binary logistic regression models used for independent analyses of associations between individual maternal PUFAs and the various allergic outcomes.After adjustment for confounders, maternal total n-3, n-6 PUFA status and the n-6:n-3 PUFA ratio were not significantly associated with offspring rhinitis, eczema, wheezing, a positive SPT and having any allergic disease with positive SPT in the

offspring (P> 0.01 for all). A weak trend of higher maternal n-3 PUFA being associated with higher risk of allergic diseases with positive SPT in offspring was observed. These findings do not support the hypothesis that the risk of early childhood allergic diseases is modified by variation in maternal n-3 and n-6 PUFA status during pregnancy in an Asian population.

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LIST OF ABBREVIATIONS

SFA	Saturated fatty acid
MUFA	Monounsaturated fatty acid
PUF	Polyunsaturated fatty acid
LA	Linoleic acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
IMDR	Acceptable macronutrient distribution range
TSLP	Thymic stromal lymphopoietin
Th	T-helper
Ig	Immunoglobulin
IL	Interleukins
IFN	Interferon
TGF	Transforming growth factor
APC	Antigen-presenting cells
COX	Cyclooxygenase
HETE	Hydroxyeicosatetraenoic acid
HPETE	Hydroperoxyeicosatetraenoic acid
PG	Prostaglandin
TX	Thromboxane
DPA	Docosapentaenoic acid
RCT	Randomized controlled trial

Chapter 1: Introduction and literature review

1.1 Introduction

Allergic diseases are one of the most common group of diseases worldwide, resulting in a significant social and economic burden(1). In most children, eczema is the earliest clinical manifestation of allergy, starting during the first few months of life. Increasing evidence shows that infants who develop allergy in early life have an altered immune response at birth(2, 3), suggesting that allergic diseases may originate *in utero*. Thus, it is now postulated that early life interventions during the antenatal period may confer protective effects on the immune system(4).

Changes in modern lifestyle, including diet, have coincided with the escalating rates of allergic diseases(5, 6). Amongst dietary factors, patters of intake of polyunsaturated fatty acids (PUFAs)have received great interest. The pro-inflammatory properties of n-6 PUFAs and anti-inflammatory properties of n-3 PUFAs are well-established in both human and animal models(7-10). For example, the n-6 PUFA arachidonic acid (AA; 20:4n-6) produces eicosanoid mediators like prostaglandin (PG)E2, which promotes the production of IgE, and leukotriene (LT)B4, which promotes airway constriction(8). In contrast, the n-3 PUFAs eicosapentanoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) act to counter the effects of AA(7). Consequently, increased intake of n-6 PUFAs and decreased exposure to n-3 PUFAs in the antenatal period have been hypothesized to increase the risk of offspring allergic diseases(11).

Fish and fish oil are sources of EPA and DHA. Fish oil supplementation studies in pregnant women(12-14) and observational studies on fish intake during pregnancy(15, 16) have suggested protective effects on offspring allergy. However, studies reporting the relationship between maternal plasma PUFA status and childhood allergic diseases have yielded inconsistent results. The Southampton Women's Survey (SWS) study found a weak protective effect of maternal EPA, DHA and total n-3 PUFAs against non-atopic

persistent/late wheezing in offspring aged 6 years(17). The KOALA Birth Cohort found AA and the ratio of n-6 to n-3 PUFAs to be protective against childhood eczema(18). No significant associations were found in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort(19) or in another small study(20). Thus, whether higher n-3 PUFA status during pregnancy would lower the risk of childhood allergic diseases remains unclear.

In the previous publications(17-20), most allergic outcome measurements were performed in Caucasian children aged 4-7 years. No study has been done in an Asian population to investigate allergic diseases at a younger age. In this study, we investigated the relationship between maternal PUFA status and potential allergic diseases up to the age of 18 months in an Asian multi-ethnic birth cohort.

1.2 Atopy and allergic disorders

1.2.1 Definitions

1.2.1.1 Atopy, allergy and allergic diseases

The nomenclature proposed in the October 2003 report of theNomenclature Review Committee of the World Allergy Organization defined atopy as a "personal and/or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens, usually proteins"(21).As a consequence, atopy is a tendency for exaggerated IgE responses. The term atopycannot be used until an IgE sensitization has been documented by IgE antibodies in serum or by a positive skin prick test (SPT)(21).

Allergy is defined as a "hypersensitivity reaction initiated by specific immunologic mechanisms"(21).Allergy refers to the clinical expression of

allergic diseases, including asthma, rhinitis, eczema and food allergy.

Allergic diseases are manifest as hyper-responsiveness in the target organ, whether skin (eczema), nose (rhinitis), lung (asthma), or gastrointestinal tract (food allergy). (Figure 1-1)

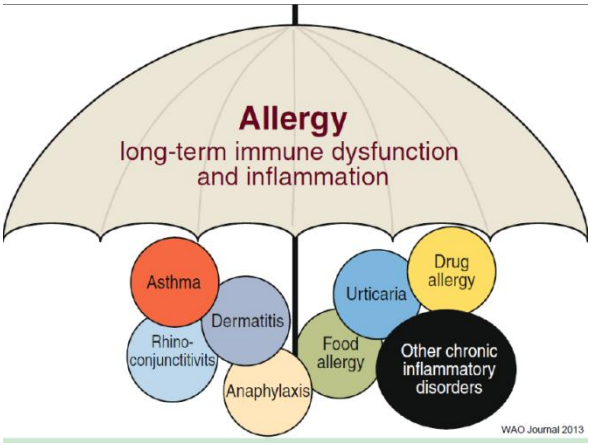

Figure 1-1Allergy and allergic diseases(22)

What makes allergy complicated is that only a proportion of atopic subjects (with a positive SPT result) have clinical symptoms (asthma, rhinitis, eczema); and those with clinical symptoms may not have a positive SPT result.Clinical symptoms are classified as non-allergic when total IgE is normal and/or specific IgE to common allergens is not detected in the serum or on skin-prick test. For example, in a whole-population birth cohort, it was reported that 30% to 40% of cases of the clinical symptoms in 4 year old children are attributableto atopy and 60% to 70% of cases could be accounted forby organ-based and other factors(23).

In addition to systematic allergy (a positive skin prick test), recent researches are exploring the potential importance of local inflammation and IgEproduction

in the mucosal tissue of the end organs. It was reported that in persistent non-allergic rhinitis, some patients may have local inflammation, nasal IgE production, and a positive response to a nasal allergen provocation test despite no evidence of systemic atopy(24). Furthermore, local allergic rhinitis (LAR) as a condition involving a localized nasal allergic response in the absence of systemic atopy has been identified(25, 26).As a consequence, although a genetic tendency of atopy may underlie all the allergic diseases, there could also be organ specific predispositions for the allergic symptoms (i.e. lower airways for asthma, nose for rhinitis and skin for eczema). In this case, different allergic diseasesmay deserve separate consideration, which will be elaborated in the following chapters.

1.2.1.2 Asthma and wheeze

Asthma is one of the most common chronic diseases of childhood, and is defined as a chronic inflammatory disease of the lower airways, leading to symptoms of recurrent wheezing and cough(27).Asthma has infancy origins and longitudinal studies found that of those children with asthma at age 7 years, about 40% have started wheezing during the first two years of life(28).

Wheezing is a high pitched, whistling sound that occur when smaller airways are narrowed by presence of bronchospasm, swelling of mucosal lining, excessive amounts of secretions, or inhaled foreign body. It is heard mostly on expiration as a result of critical airway obstruction(29).The Tucson Children's Respiratory Study, a prospective birth cohort studies starting in 1980, proposed three different patterns of recurrent wheezing in pediatric patients(30): transient

early wheezing, non-allergic wheezing, and allergic wheezing (31). Transient infant wheezing is relatively benign and most children would stop wheezing after the age of 3 years. Non-allergic wheezing is mainly triggered by viral infection and tends to remit later in childhood. Allergic wheezing is linked to IgE-mediated sensitization. It includes early atopic wheezingand late atopic wheezing.Early atopic wheezingtakes the most part ofwhat we have called in the past ‘persistent wheezing’. Late atopic wheezingis what we called in the past‘late-onset wheezing’, and the patients only started wheezing at 6 years of life.

1.2.1.3 Rhinitis

Rhinitis is an inflammation of the upper airways that is characterized by symptoms of runny (rhinorrhea) and/or blocked nose and/or sneezing occurring for two or more consecutive days and lasting for more than an hour for most days (32, 33).Diary recording of symptoms and their circumstances over a 2-week period may be helpful in borderline cases. Though not viewed as life threatening, rhinitis impairs quality of life, sleep, work (34) and school performance(35), and have the long-term risk ofincreasing the development of asthma (36).

From an etiologic point of view, noninfectious rhinitis has been traditionally classified as allergic rhinitis (AR) and nonallergic rhinitis (NAR) based on the presence and absence of allergic sensitization(32).However, this approach has recently been suggestedto be incomplete because patients previously given a diagnosis of NAR might actually be classified as having Local allergic rhinitis

(LAR) because they have nasal symptoms after Nasal allergen provocation test (NAPT) with a common aeroallergen (24, 37), and local production of sIgE was detected in these patients. LAR is a localized nasal allergic response in the absence of systemic atopy characterized by local production of specific IgE (sIgE) antibodies, a TH2 pattern of mucosal cell infiltration during natural exposure to aeroallergens, and a positive nasal allergen provocation test response with release of inflammatory mediators (tryptase and eosinophil cationic protein) (25).As a result, a new etiological classification of rhinitis has been proposed (Table 1-1)(25, 38).However, it remains a matter of debate whether local sensitization would be the primary event in any AR disease and can develop into systemic classical AR in the future (25). This requires appropriate prospective studies.

Table1-1 Etiologic classification of rhinitis.

1. Allergic rhinitis
➢ **Allergic rhinitis (with systemic atopy)**
i. Classical classification
1. Time of exposure to aeroallergen or aeroallergens: perennial, seasonal, and occupational
ii. ARIA classification(32)
1. Duration of symptoms: persistent and intermittent
2. Severity of symptoms: mild, moderate, and severe
➢ **Local allergic rhinitis (without systemic atopy)**
i. Classical classification
1. Time of exposure to aeroallergen or aeroallergens: perennial, seasonal, and occupational
ii. ARIA classification(32)
1. Duration of symptoms: persistent and intermittent
2. Severity of symptoms: mild, moderate, and severe
2. Nonallergic rhinitis
➢ **Infectious**
➢ **Occupational (irritant)**
➢ **Drug induced**
➢ **Hormonal**
➢ **Irritant**
➢ **Food**
➢ **Emotional**
➢ **Atrophic**
➢ **NARES**
➢ **Idiopathic**

Adapted from Rondon et al. (38)

1.2.1.3 Eczema

Eczema is a chronic inflammatory pruritic skin disease that affects a large number of children and adults in industrialized countries (39). It often begins in early infancy and follows a course of remissions and exacerbations(40), thus is considered to be one of the first manifestations in the atopic march. 50% of those with eczema during the first 2 years of life will develop asthma subsequently (41). The severity of eczema, including early sensitization to food, increases the risk of asthma and allergic rhinitis(40, 42).Infants typically present with erythematous papules and vesicles on the cheeks, forehead, or scalp, which are intensely pruritic(39).Scoring Atopic Dermatitis (SCORAD) (43) has been used to classify AD into 3 main severity forms: mild (<15), moderate (>15 and <40) and severe (>40).

Eczema has been subtyped as allergic (formerly extrinsic) and nonallergic (formerly intrinsic), representing approximately 80% and 20% of adult patients, respectively (44).The term topic eczema is used when the underlying inflammation is dominated by an IgE-antibody associated reaction, determined based on an IgE-antibody determination or skin test. Otherwise it should be termed non-atopic eczema(21).

1.2.2 The allergic march

A pattern of progression through different allergic disorders in early childhood has been termed the ‘allergic march’, with eczema and food allergy dominating in early childhood, while asthma and rhinitis are more common later (45). (Figure 1-2)

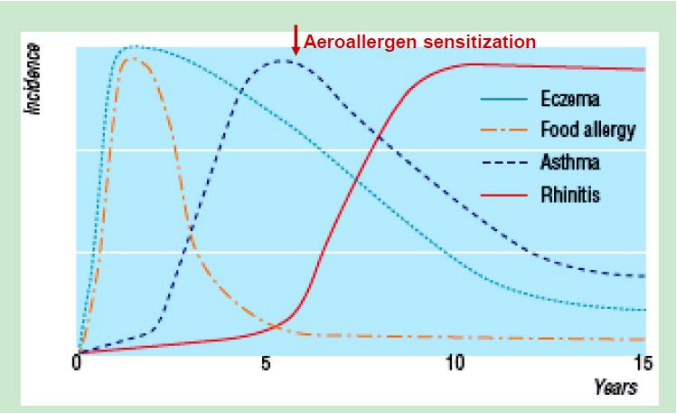

Figure1-2 Incidences of different types of allergic diseases by age.(22)

Evidence for the allergic march from eczema to allergic rhinitis and asthma are raised from longitudinal studies. Rhodes et al. (46, 47)followed 100 infants with at least one allergic parentup to 22years in the United Kingdom. The prevalence of eczema peaked at 1 year of agein 20% of children,but later declined to approximately 5% at 22 years of age. However, the prevalence of allergic rhinitis slowly increased over time, from 3% to 15%. The prevalence of parents reporting wheezing increased from 5% at the age of 1 year to 40% at 22 years of age. Moreover, sensitization to allergen tested by skin prick test increased over time to a peak of 36% at 22 years of life. The Tucson Children's Respiratory Study found that eczema during the first 2 years of life was an independent risk factor for persistent wheezing up to 6 years of life, and was associated with inactive and chronic asthma but not with newly diagnosed asthma at 22 years old(30, 48).

The putative mechanism of the allergic march is that the allergen exposure through the epidermis can initiate systemic allergy and predispose individuals

to allergic rhinitis, and asthma in the airways(45, 49). Epithelial barrier defects derived from loss-of-function mutations in the filaggrin gene have been identified as a strong predisposing factor for eczema and secondarily, to the development of asthma(50). Thymic stromal lymphopoietin (TSLP), apro-inflammatory factor derived from epithelial cells have also elicited considerable interest, asit has been shown to stimulate mast cells to produce TH2 cytokines(51).

1.2.3 Fetal and early origin of allergic diseases

The “development origins of health and disease” paradigm maintains that nutritional or other environmental stimuli during critical periods of growth and development have the potential to permanently “program” the structure and/or function of cell populations, emerging organ systems, or homeostatic pathways (52). Since Barker’s findings that exposures in utero could have lifelong influenceon cardiovascular diseases and othertraits (53), there has been considerable interest in the role of early life events plays in health and diseases.

Early life origins of asthma have been recognized in birth cohort studies.Children who have a diagnosis of asthma have often started wheezing during infancy. Indeed early age of onset is a recognized risk factor for persistence of asthma(48, 54). The ALSPAC study which includes 6265 children found thatof the children who have asthma at 7 years of age, about 40% have started wheezing during the first two years of life(28).Other longitudinal birth cohortshave shown strong association between lung function(55) and airway responsiveness(56) measured soon after birth and asthma later in

childhood. Furthermore, cohorts followed from childhood to adult life (57)have demonstrated that lung function changes associated with asthma become established in early childhood and then track to adulthood. Theseresultslead to the hypothesis that pulmonary developmental changes associated with asthma in childhood and even adulthood are already established at birth or shortlyafter that (3).On the other hand, fetal exposure to environmental factors such as maternal smoking, diet have been reported to be linked to the development of the fetal immune system(58),decreased lung function(59), and risk of developing asthma and wheezing in the offspring(60, 61). These evidences strengthen the hypothesis the fetus is not immunologically naive and intrauterine exposures can act directly to invoke immunological sensitization leading to postnatal airway inflammation.

1.3 Polyunsaturated fatty acid (PUFA)

1.3.1 Definition and nomenclature

There are three kinds of fatty acid: saturated (SFA), monounsaturated (MUFA, possessing one carbon-carbon double bond), or polyunsaturated (PUFA, possessing two or more carbon-carbon double bond).

The standard numbering system for fatty acids gives the number of carbon atoms, the number of double bonds (after a colon), and the position of the first double bond (after the letter n) counting from the end of the carbon chain opposite the carboxyl group. For example, linoleic acid (LA) is denominated as 18:2n-6, because it has a total of 18 carbon atoms in the chain, with 2 double bonds, and the first double bond is on the 6^{th} carbon position from the methyl. In

addition, fatty acids are often expressed by their abbreviations. The fatty acids relevant to the current thesis are listed as follows:

- ✓ Linoleic acid (LA; 18:2n−6)
- ✓ Arachidonic acid (AA; 20:4n−6)
- ✓ α-Linolenic acid (ALA; 18:3n−3)
- ✓ Eicosapentaenoic acid (EPA; 20:5n−3)
- ✓ Docosahexaenoic acid (DHA; 22:6n−3)

1.3.2 Categories and biosynthesis of PUFAs

The number, position,and configuration of the double bonds of PUFAs also largely determine their physicaland biologic properties. Biologically relevant families of PUFAs are the n–6 and the n–3 fatty acids. In the n-6 PUFA family, LA is the simplest member and as the precursor of n-6 family PUFAs, it is capable of being metabolized to longer-chain, more unsaturated n-6 PUFAs. LA is first converted to γ-linolenic acid (18:3 n–6) by Δ6-desaturase, and thenγ-linolenic acid can be elongated (by elongase) to dihomo-γ-linolenic acid (20:3 n–6). Dihomo-γ-linolenic acid canbe desaturated further by Δ5-desaturase, yielding AA. Similarly, ALA is the simplest members of n-3 family PUFAs and can be synthesized to a sequence of longer chain n-3 fatty acids, including EPA and DHA. (Figure 1-3) During this process, n-3 and n-6 PUFAs are competing for the same set of enzymes, such as δ-6 desaturase. Although supplemental ALA raises EPA and DPA status in the blood, ALA or EPA dietary supplements have little effect on blood DHA levels(62). This result demonstrates that the rate of conversion from ALA to DHA is very low in human.

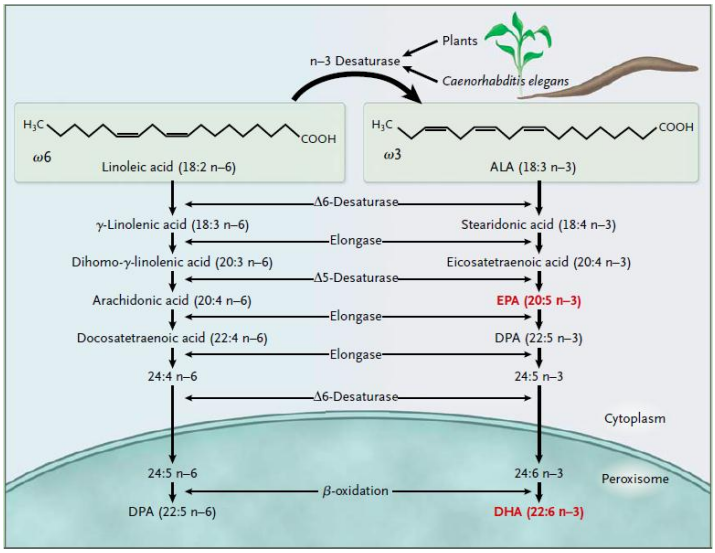

Figure 1-3The biosynthesis of n−6 and n−3 polyunsaturated fatty acids. LA and ALA can be synthesized to more unsaturated PUFAs, during which process they are competing for the same set of enzymes.EPA and DHA, the most biologically relevant n–3 fattyacids, are highlighted in red.(63)

1.3.3 Requirements and changing in intakes for PUFAs

LA and ALA cannot be synthesized in mammals and human, as mammals lack enzymes to insert thedouble bond in the n–6 or n–3 position.Therefore they are defined as essential fatty acids to human. The lack of LA and ALA, as well as some of their elongatedand more unsaturated products, leads to a syndrome of deficiency (64, 65).This syndrome of deficiency is usually characterized by desquamativerashes and hyperkeratoticdermatoses in humans. Current estimates of the minimum requirementsfor n–6 and n–3 fatty acids in adults are 1.0% and 0.2% of daily energy intake,respectively. (66)An expert consultation of FAO and WHO recommended that for adult males and non-pregnant/non-lactating adult females the acceptable macronutrient distribution range (IMDR) of DHA plus EPA should be0.25 to 2.0 g per day. For adult pregnant and lactating females, the minimum intake for optimal adult health and fetal and infant development is 0.3 g/d EPA+DHA, of which

at least 0.2 g/d should be DHA. There is insufficient evidence to set a specific minimum intake of either EPA or DHA alone; both should be consumed(66).

LA is found in significant quantities in many vegetable oils, including corn, sunflower, and soybean oils, and in products made from such oils, such as margarines. AA is found in meat and offal and intakes are estimated at 50 to 500 mg/day. EPA, DPA, and DHA are found in fish, especially so-called “oily” fish (tuna, salmon, mackerel, herring, and sardine). One oily fish meal can provide between 1.5 and 3.5 g of these long-chain n−3 PUFAs. Fish oils supplements available in the commercial market contain 30% long-chain n−3 PUFAs of the fatty acids in the capsule. Thus, consumption of a typical 1-g fish oil capsule per day can provide about 300 mg of these fatty acids. In the absence of oily fish or fish oil consumption, intake of long-chain n−3 PUFAs is likely to be <100 mg/day, although foods fortified with these fatty acids are now available in many countries (15). In the United States, intake of n-3 fatty acids EPA and DHA is only 0.1–0.2 g/d (67), which is below the recommendation of 0.2g/d byFAO and WHO(66).

In 20th century, the amount of linoleic acidin western diet has increased remarkably, with the change being most marked since the early 1960s. The availability of linoleic acid (LA) increased from 2.79% to 7.21% of energy from 1909 to 1999 in the United States(68). These changes are in large part due to a significant increase in the use of margarine and vegetable oils, which contain large amount of LA. Although from 1909 to 1999, the availability of n-3 PUFA ALA increased 85% from 0.39% of energy to 0.72% of energy, there were no

remarkable changes in the availability of long-chain n-3 PUFAs EPA, DPA and DHA (68). As a result, the ratio of n-6 to n-3 fatty acids is around 9.8:1 at the end of 20th century (67). This biased intake that favors n-6 PUFAs intake has been linked to the increased prevalence of a variety of diseases such as cardiovascular diseases (63)and allergic diseases(69).

1.3.4 Biomarkers of PUFAs

Accurate assessment of PUFA intake is essential to examine the associations between PUFAs in diet and disease risk in epidemiological research. However, relative intakes of individual PUFAs in the diet are difficult to estimate accurately from dietary assessment methods such as food frequency questionnaires food recall and food diary. This is in part because that respondents often under-report consumption, especially in obese population (70). Moreover, respondents would consciously or sub-consciously alter their usual diet, during the recording period. Interviewer bias and respondent burden also add to the imprecise measurement. Instead, using plasma fatty acids concentrations as a biomarker for dietary intake can complement the drawbacks dietary assessment methods, and have the potential to be used more quantitatively (71). It seems reasonable to expect that the best markers of dietary intake exist for the fatty acids that cannot be endogenously synthesized. These include the n-3 PUFAs (ALA from plant sources and long-chain n-3 fatty acids from marine sources), the n-6 polyunsaturated fatty acids (mostly from vegetable oils).

Adipose tissue, plasma lipid fractions (such as plasma total phospholipids and

phosphorylcholine), and erythrocyte total phospholipids are the three types of human sample that are often used as biomarkers of PUFAs. The fatty acid composition of adipose tissue has been considered a gold standard for the representation of dietary fatty acids, due to the slow turnover time in weight stable individuals. The $t_{1/2}$ of adipose tissue lipids were estimated to be between 6 and 9 months using stable isotope methodology (72). Significant positive correlations between the relative intake of dietary PUFA and the relative content of adipose tissue n-6 and /or n-3 or total PUFA have been noted (71). Plasma lipid fractions can reflect only recent, that is the preceding few weeks, rather than long-term intake. Within days after altered composition of dietary fatty acids, the fatty acid composition of plasma lipid fractions change accordingly (73). Traditionally, fatty acids measured in erythrocytes were thought to represent fatty acid intake for several months, because erythrocytes have a life span of approximately 120 days. However, it has been reported that the fatty acid composition of erythrocyte PL reflects changes in dietary fatty acid intake within 24 h with an increase of LA (73) and this could only be explained by the exchange and transfer of fatty acids from plasma to erythrocytes. In this case, fatty acids measured in erythrocytes are representing a period of fatty acid intake as short as that in plasma lipid fractions.

1.4 Mechanisms linking PUFA and allergy

1.4.1 Mechanisms of allergy

The immunological mechanism associated with allergy is the biased expression of T-lymphocyte and cell-mediated responses to common allergens towards T-helper-2 (Th-2) lymphocyte activity. Th-2 lymphocytes give rise to peptide

regulatory factors named cytokines. The Th-2 cytokinesinitiate the production of IgE, the primary allergy-promoting antibody, and activate inflammatory cells such as eosinophils, which are commonly associated with allergic inflammation. TheseTh-2 cytokines include interleukins (IL)-4, IL-5, IL-9 and IL-13. The counter-regulatory pathways include those generated by a normal immune response to infection dominated by the T-helper-1 (Th-1) lymphocytes. Th-1lymphocytes generate the cytokines interferon-gamma (IFN-γ) and IL-2. Another pathway involves a group of T-lymphocyte regulators which have an influence on both Th-1 and Th-2 activity either by cell-cell contact or by the generation of IL-10 and transforming growth factor (TGF)-β. Based on this mechanism it becomes clear that either overexpression of Th-2 activity or a failure of control by Th-1 or T-regulatory function will result in a higher probability of the development of allergy and allergic inflammation. The pattern of response of T-lymphocytes is dictated by the nature of the signaling from antigen-presenting cells (APCs). They in turn are affected by the nature of the antigen exposure. APCs generate IL-12, -15, -18 and -23 which predominantly stimulate Th-1 responses, whereas IL-10 from regulatory T-cells inhibits IL-12 and therefore favors Th-2 activity.

1.4.2 n-6 fatty acidsand allergic inflammation

It has been suggested that there is a causal relationship between the increased intake of the n−6 PUFA LA over the latter part of the twentieth century and allergic disease (5). The key link between PUFAs and allergy lies in the eicosanoids generated from AA. AA in the inflammatory cell membrane phospholipids is the major precursor of for eicosanoid synthesis. (Figure 1-4)

Eicosanoids, which include prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs) and other oxidized derivatives, are generated from AA by the action of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes within seconds to minutes of acute challenge of immune system. PGs and LTs are are widely appreciated for their pro-inflammatoryactivities(74, 75). For example, PGD2, which is produced mainly by mast cells and activated macrophages, is a potent bronchoconstrictor, promotes vascular permeability, and activates eosinophils and a Th2-type response. LTB4 is chemotactic for leukocytes, increases vascular permeability, induces the release of lysosomal enzymes and reactive oxygen species by neutrophils and of inflammatory cytokines (e.g., TNF-α) by macrophages, and promotes IgE production by B cells (15). The eicosanoids frequently have opposing effects (76).For example, although PGE2 is well known for its pro-inflammatory property to inhibits the production of Th1-type cytokines and primes naïve T cells to produce IL-4 and IL-5, and promote the production of IgE(8, 74), it has also been found to have anti-inflammatory properties by promoting the formation of lipoxins, which is involved in the resolution of inflammation(69, 77). Thus, the overall physiologic (or pathophysiologic) outcome will depend on the nature of cell types present and the nature, timing and duration of the stimulus.Table 1-2 summarized the pro- and anti-inflammatory effects of PGE2 and LTB4.

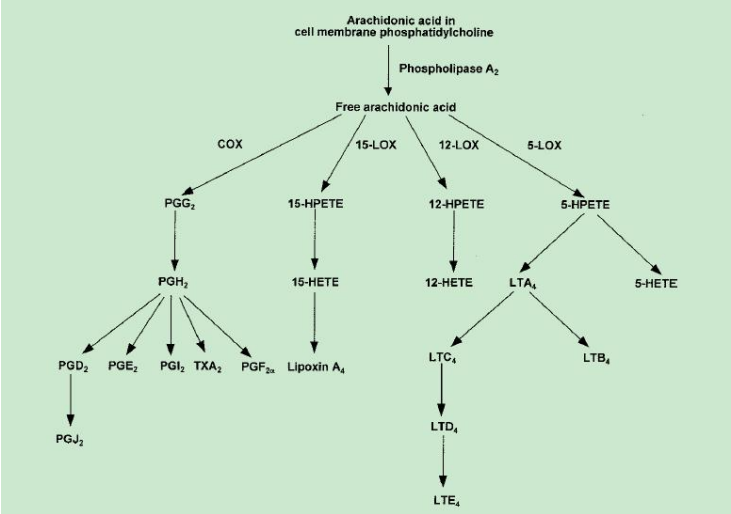

Figure 1-4 Generalized pathway for the conversion of arachidonic acid to eicosanoids. COX, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane.(69)

Table 1-2 Pro- and anti-inflammatory effects of PGE2 and LTB4.

PGE2
➢ **Pro-inflammatory**
Induces fever
Increases vascular permeability
Increases vasodilatation
Causes pain
Enhances pain caused by other agents
Increases production of IL-6
➢ **Anti-inflammatory**
Inhibits production of TNF and IL-1
Inhibits 5-LOX (decreases 4-series LT production)
Induces 15-LOX (increases lipoxin production)
LTB4
➢ **Pro-inflammatory**
Increases vascular permeability
Enhances local blood flow
Chemotactic agent for leukocytes
Induces release of lysosomal enzymes
Induces release of reactive oxygen species by granulocytes
Increases production of TNF, IL-1, and IL-6

1.4.3 n-3 fatty acids and allergic inflammation

N-3 PUFAs are potentially potent anti-inflammatory agents.This property of n-3 fatty acids were first postulated in the study of Greenland Eskimos (Inuits), whose diet was composed mainly of seal and whale and was extremely rich in marine n–3 fatty acids. A lower frequency of bronchial asthma, together with

other chronic diseases such as myocardial infarction was reported in Inuits, as compared with Danish controls (78).

n−3 PUFAs, such as EPA and DHA act to counter the effect of n−6 PUFAs by competing for the same desaturase enzymes used to produce AA, and partly replacing AA in inflammatory cell membrane in a dose-response pattern(7).Because less substrate of AA is available for eicosanoids production,n-3 PUFAs can decrease the pro-inflammatory eicosanoids produced by inflammatory cells, such as PGE2 (79, 80), LTB4(81, 82), and 5-hydroxyeicosatetraenoic acid (81, 82).On the other hand, EPAin inflammatory cells can also be metabolized into eicosanoids, such as LTB5, LTE5, and 5-hydroxyeicosapentaenoic acid(81, 82). (Figure 1-5**)** However, theseeicosanoids are in general much less potent local mediators than the corresponding n–6 fatty acid derivatives(69).For example, LTB5 is 10- to 100-fold less potent as a neutrophil chemotactic agent than LTB4 (83).

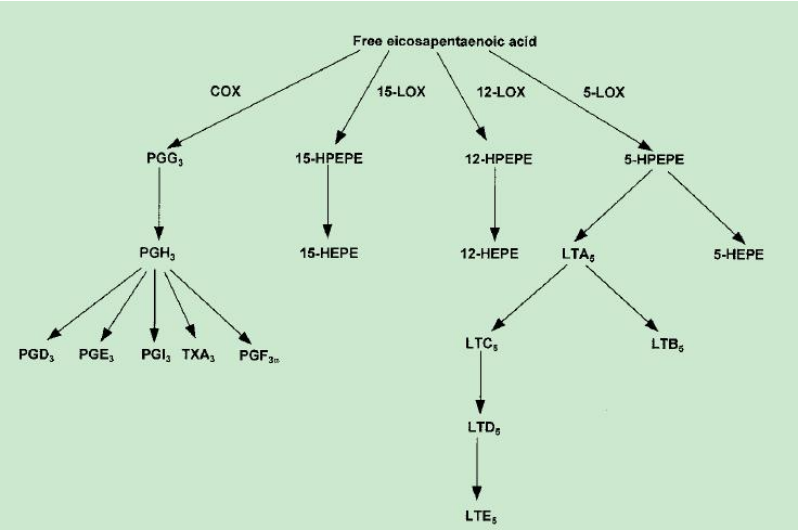

Figure 1-5 Generalized pathway for the conversion of eicosapentaenoic acid to eicosanoids.COX, cyclooxygenase; HEPE, hydroxyeicosapentaenoic acid;HPEPE, hydroperoxyeicosapentaenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane.(69)

Recent research based on lipidomics and informaticsidentified a new family of dual anti-inflammatory and pro-resolution lipid mediators termed resolvins, which are derived from long-chain n-3 PUFAs. (77)They are so-named asthey proved to be potent regulators of resolution. The two chemically unique structural forms of resolvins, E-series and D-series are derived from EPA and DHA, respectively.The biosynthesis pathways of resolvins are showed in Figure 1-6. For example, EPA can be converted to 18R-hydroperoxyeicosapentaenoic acid (18R-HPEPE) and rapidly transformed by activated human neutrophil 5‑lipoxygenase to Resolvin E1and E2. E‑series member resolvin E1 reduces inflammation and blocks human neutrophil transendothelial migration, thereby displaying potent anti-inflammatory actions.(84)

DHA in resolving exudates is also converted to another molecule belonging to a new family of mediators named protectins.Protectin D1 blocks T‑cell migration in vivo, reduces TNF and interferon‑γ secretion and promotes T‑cell apoptosis. (85)

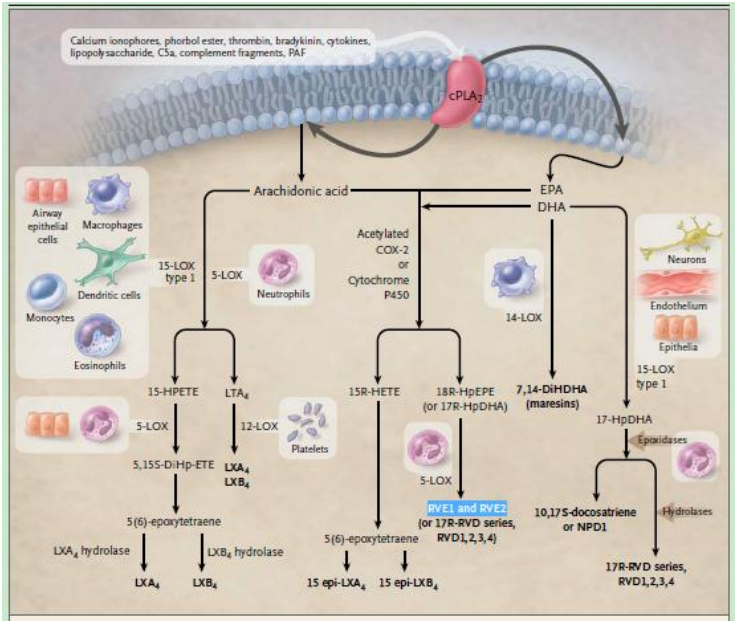

Figure 1-6 Biosynthesis of resolvins and protectins from DHA and EPA.COX-2 denotes cyclooxygenase-2, cPLA2 cytosolic phospholipase A2, DHA docosahexaenoic acid, DiHDHAdihydroxy-docosahexaenoic acid, DiHp-ETE dihydroperoxy-eicosatetraenoic acid, EPA eicosapentaenoic acid, HETE hydroxy-eicosatetraenoic acid, HpDHAhydroperoxy-docosahexaenoicacid, HpEPEhydroperoxy-eicosapentaenoic acid, HPETE hydroperoxy-eicosatetraenoic acid, 5-LOX 5-lipoxygenase, 12-LOX 12-lipoxygenase,14-LOX 14-lipoxygenase, 15-LOX 15-lipoxygenase, LTA4 leukotriene A4, LXA4 lipoxin A4, LXB4 lipoxin B4, NPD1 neuroprotectin D1,and RV resolvin.(63)

Other possible mechanisms of anti-inflammatory property of n-3 fatty acids might include reducing the capacity of Atigen Presenting Cells to present antigen to T cells, reducing T cell proliferation, influencing T regulatory cells (11).

1.5 Literature review

1.5.1 Cohorts of maternal PUFA status and offspring allergy

Cohorts examining the relationship between maternal PUFAs status and offspring allergic outcomes have reached mixed results. (Table 1-3) The large ALSPAC cohort (19) found no relation between maternal red cell PUFAs and

wheezing and eczema before 4 years of age, and a small study by Yu and Bjorksten(20) found no association between maternal serum PUFAs and offspring atopic outcomes to 6 years of age. The SWS study (17)reported a modest protective effect of DHA, EPA and total n-3 PUFAs against non-atopic persistent wheezing up to 6 years of age, but not on other phenotypes of wheezing.Furthermore, the authors accepted that the chance of false positive finding cannot be excluded, as numbers of associations were tested. The KOALA Birth Cohort (18)unexpectedly reported a protective effect of AA against eczema in the first 7 months of life, and the ratio of n-6 to n-3 PUFAs against eczema in 6-7 years children. This is against the widely held notion that excessive AA and a high ratio of n-6 to n-3 PUFAs might increase the risk of allergic disease. (5, 69)

494 Table1-3Summaries of studies of maternal fatty acid status and allergic
495 outcomes in infants and children.

Reference and cohort	Exposure	Outcome measures and confounding factors	Findings
(19) ALSPAC	Red cell PL PUFA in late pregnancy (after 20 weeks of pregnancy)	Wheezing at 0 to 6 mo and 30 to 42 mo (n=1191); Eczema at 18 to 30 mo(n=1238) Confounding: child's sex, gestational age at birth, and birth weight, mother's age, education level, housing tenure, parity, ethnicity, smoking in pregnancy, maternal atopic disease, child's head circumference at birth, child's crown to heel length at birth, mother's body mass index, breast-feeding in first 6 months, and day care use in first 6 months.	No significant result was found between maternal PUFA status and offspring transient wheezing, later-onset wheezing, persistent wheezing, and eczema.
(18) KOALA	plasma PL PUFA at 34–36 weeks of pregnancy	Wheeze, asthma, allergic rhinoconjunctivitis, eczema, atopic dermatitis, allergic sensitization, and high total IgE until the age of 6–7 years (n=1275) Confounding: recruitment group, age of the mother, maternal ethnicity, maternal education level, maternal smoking during pregnancy, parental history of atopy and/or asthma, presence of older siblings, term of gestation, season of birth, gender, birth weight, mode of delivery, child exposure to environmental tobacco smoke, breastfeeding, child day care, and pets at home	High ratio of maternal n-6 vs. n-3 LCPs was associated with a lower risk of eczema in the child (P for trend 0.012). More specifically, a decreased risk of eczema in the first 7 months of life with increasing AA levels (P for trend 0.013) was reported. No associations were found between maternal fatty acids and offspring airway-related atopic manifestations, sensitization, or high total IgE.
(17) SWS	34wk of gestation	Airway inflammation; wheezing at 6, 12, 24, and 36 mo and 6 years (transient, persistent, late-onset wheezing); SPT, fractional exhaled nitric oxide (FENO) measurement, and spirometry at 6 years. (n=865) Confounding: maternal asthma and rhinitis, parity, paternal asthma, maternal smoking in pregnancy, child's sex, and maternal educational attainment, maternal smoking during pregnancy, and dogs/cats in the home during the child's infancy.	Higher maternal EPA, DHA, and total n-3 fatty acids were associated with reduced risk of nonatopic persistent/late wheezing (RR=0.57, 0.67 and 0.69, resp. P = 0.01, 0.015, and 0.021, resp.).A higher ratio of linoleic acid to its unsaturated metabolic products was associated with reduced risk of skin sensitisation (RR 0.82, P = 0.013).

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1.5.2 RCTs of maternal fish oil supplementation and offspring allergy

SixRCTs were identified studying the effect of maternal fish oil supplementation during pregnancy onallergic outcomes in the offspring.Krauss-Etschmann et al. (86) reported that higher levels of DHA and EPA in both maternal andcord blood in group fish oil supplementation. Dunstan et al. (12)showed a higher DHA and EPA status in cord blood erythrocytes. These results demonstrated the effect of fish oil supplementation on elevating maternal and neonatal n-3 PUFA status. These studies also showed immunology changes towards a balanced Th1/Th2 response, such as lower cord blood plasma IL-13, more IL-5 responsive colony forming units, lowerIL-10 in response to cat allergen(12, 87-91)higher TGF-β mRNA, lower IL-4, IL-13 and CCR4 mRNA(86)in cord blood. These immunologiceffects of fish oil supplementation might have effect on allergic sensitization,and in turn on the development of allergic diseases in theoffspring.Dunstan et al. (12) reported a lower risk of a positive SPT to egg in fish oil group. Clinical outcomes of allergic diseases such as less severity of eczema (12), lower prevalence of food allergy and IgE associated eczema (14), asthma, allergic asthma, allergic diseases (13) were reported in the fish oil supplementation group. However, a recent study in Australia did not report any protective effect of fish oil supplementation on allergic sensitization or IgE associated allergic diseases (92).

Table 1-4Summaries of studies of maternal fish oil supplementation during pregnancy and allergic outcomes in infants and children.

Reference	Study design	Outcome measures	Findings
(12, 87-91)	Perth,Australia Doubleblinded RCT Subjects: 83 atopicpregnantwomen FO:2.2g DHA, 1.1g EPA; n=40 Control: oliveoil; n=43 From week 20 of pregnancy until delivery	**Clinical assessments:** SPT at age 1 year Clinical symptoms of allergic diseases (asthma, wheeze, food allergy,atopic dermatitis) at 1 year **In cord blood:** Plasma cytokine(Th2: IL-4, IL-5,IL-13, TNF-α; Th1: IFN-γ,IL-12,Treg: IL-10; Th17: IL-6,) APC function (HLA-DRexpression and cytokineresponses) Mononuclear cell cytokineresponses to allergens andmitogen (IL-5, IL-10, IL-13,IFN-γ) Plasma total IgE $CD34^{+}$cell numbers CD34+ cell expression ofcytokine (IL-5Rα, IL-3Rα) orchemokine(CXCR4, CCR3) receptors. Eosinophil/Basophil colonyforming units Leukotriene production bystimulated neutrophils **In breast milk (3 days postpartum):**Immunomodulatoryfactors - sCD14, IgA,cytokines (IL-5, IL-6, IL-10,TNF-α and IFN-γ)	**FO associated with:** Lower risk of a positive SPT to egg (OR 0.34, 95% CI 0.11–1.02; p=0.055) Less severity in infants with atopic dermatitis (OR 0.09, 95% CI 0.01–0.94; p=0.045) Lower cord blood plasma IL-13 (p<0.05) Lower mononuclearcell cytokine responses(onlyIL-10 in response to catallergen is statistically significant; p=0.046) A higher percentage of cordblood CD34+ cells (p<0.002) More IL-5 responsive colonyforming units (p<0.003) Lower neutrophil LTB4 production (p=0.031)
(86)	Multicenter: Granada,Spain; Munich,Germany; Pecs, Hungary Doubleblinded2-factorialRCT Subjects: 311 pregnantwomen 4 groups: 1. FO: 0.15 g EPA+0.5 g DHA/day n=45 2. 5-MTHFn=49 3. FO+5-MTHFn=49 4. Control: plainmilk basedsupplementn=50 From week 22 of pregnancy until delivery	**In maternal and cord blood at birth:** Th1/Th2 related molecules: mRNAexpression of CCR4, IL-13,IL-4, CRTH2, CXCR3, IFN-γ, IL-1, TGF-β **In cord blood:** Lymphocyte subsets	**Maternal FO was associatedwith:** Higher TGF-β mRNA inmaternal and cord blood (both p<0.001) Lower IFN-γ and IL-1 mRNAin maternal blood (all p<0.001) Lower IL-4, IL-13 and CCR4mRNA in cordblood (both p<0.001) Lower proportions of NK cellsand CCR3+ CD8+ T-cells incord blood (p<0.001and p<0.04, respectively)
(13, 14, 93)	Linkoping,Sweden Double blinded RCT	**Clinical examinations ofinfants:** Skin prick testing to cow'smilk, egg, and wheat at 6 and12 months of age	**Maternal FO was associated with:** Lower prevalence of foodallergy (2% vs. 15% in controlgroup; p<0.05)

	Subjects: 145 pregnantwomen withallergicfamily history FO:21.6 g EPA+1.1 g DHA/day; n=52 Control: soybean oil; n=65 From week 25 of pregnancy until end of lactation (3-4 months of breastfeeding)	Plasma specific IgE to egg/milk/wheat at 3 and12 months age IgE associated eczema and foodallergy at 3, 6, and 12 monthsof age **In maternal wholeblood:** Production of eicosanoids(PGE2, LTB4), cytokines(IFN-γ, IL-5, IL-6, TNF, IL-8,IL-10) and chemokines(CCL2, CCL3) by LPSstimulated maternal wholeblood cultures	Lower prevalence of IgEassociatedeczema (8% vs. 24% in control group; p<0.05) LPS-induced PGE2 secretiondecreased in 64% of the FOsupplementedmothers andincreased in 77% of those in thecontrol group (p=0.002). Thedecreased PGE2 productionwas more pronounced amongnon atopic (80%) than atopicmothers (69%) (notsignificant). LPS-inducedcytokine and chemokinesecretion was not affected
(13)	Copenhagen, Denmark Doubleblinded RCT Subjects: 533pregnantwome n 3 groups: FO:1.1g DHA, 1.6g EPA; n=266 Control: oliveoil; n=136 No oil capsulesn=131 From week 30 of pregnancy until delivery	**Clinical examinations at 16 years of age:** Asthma Allergic asthma, Asthma ofmixed type, atopic dermatitisor allergic rhinitis Allergic asthma, atopic dermatitis or allergic rhinitis data takenfrom the National patientregistry in Denmark	**Maternal FO was associated with:** Lower risk of asthma (OR=0.37,95% CI 0.15–0.92, p=0.03) Lower risk of allergic asthma(OR=0.13, 95% CI 0.03–0.60, p=0.01) Lower risk of asthma of alltypes, atopic dermatitis orallergic rhinitis (OR=0.43,95% CI 0.19-0.96, p=0.04) Lower risk of allergic asthma,atopic dermatitis or allergicrhinitis (OR=0.31, 95% CI0.11–0.84, p=0.02)
(92)	Adelaide, Australia. DoubleblindedRC T Subjects: 706 pregnant women with allergic family history FO:0.8g DHA, 0.1g EPA; n= 368 Control: vegetable oil; n=338 From 21 weeks' gestationuntil birth	SPT to at least one allergen at 1 or 3 years of age IgEmediatedallergic disease	No significant differences were found No significant differences were found

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1.6 Study hypothesis and aims of study

Hypothesis: Increased pro-inflammatory n-6 PUFA status and reduced anti-inflammatory n-3 PUFA status in pregnant women are associated with increased risk of childhood allergic diseases.

Aim of the study: to examine the association of maternal PUFA status measured in plasma samples (percentage of total n-3 PUFAs, total n-6 PUFAs, n-6 to n-3 PUFA ratio, and the specific n-6 and n-3 PUFAs)with clinical outcomes of potential allergic diseases (rhinitis, eczema, and wheezing), results from skin prick testing (SPT) and allergic diseases (SPT plus clinical outcomes) in 18 months old children.

Chapter 2. METHODS

2.1 Participants

Participants were mother-child pairs in the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) birth cohort. A detailed study profile has been described elsewhere(94, 95). In brief, the GUSTO study is designed to investigate the role of early life exposures in the development of metabolic and other diseases. Between June 2009 and September 2010, 1162 pregnant women aged 18 years and above were recruited in the main GUSTO. The study was granted ethical approval by the Institutional Review Board of the KK Women's and Children's Hospital (KKH) and National University Hospital (NUH). Informed written consent was obtained from each participant.

Detailed interviews of maternal characteristics, including demographics, lifestyle, diet and health, were conducted at a recruitment clinic visit and again at 26–28 weeks of gestation. Infant characteristics, such as fetal anthropometry and health outcomes, were collected through examination at home at 3 weeks, 3 months and every 3 months thereafter until 15 months of age. At the age of 18 months, the mothers and infants were invited to the study clinic for detailed clinical assessment including allergic sensitization (skin prick testing).

2.2 Maternal plasma polyunsaturated fatty acid (PUFA)

Blood was taken into heparinized tubes at 26-28 weeks of gestation. Plasma was prepared and stored at -80 ℃ until analysis. Plasma lipids were extracted with chloroform/methanol (2:1 vol/vol). Phosphatidylcholine (PC), which contributes about 75% of plasma phospholipid, was isolated by solid phase

extraction. Then, fatty acid methyl esters (FAMEs) were generated from PC after reaction with methanol containing 2% (vol/vol) sulphuric acid. FAMEs were extracted into hexane and separated by gas chromatography(Series 6890, Hewlett Packard, BPX 70column SGE Europe Ltd.). FAMEs were identified by comparison with retention times of standards run previously and they were quantified using ChemStation software(Agilent Technologies). Data was expressed as percentage contribution to the total plasma PC fatty acid pool. For all fatty acids within plasma PC, within-assay CV is < 3% and between assay CV is < 6%. In this study, we focused on percentage of total n-3 PUFA, total n-6 PUFA, and n-6 to n-3 fatty acid ratio. Additionally, we examined the specific PUFAs, specificallyALA, EPA, docosapentaenoic acid (DPA; 22:5n−3), DHA, EPA+DHA, LA, and AA.

2.3 Allergy outcome measurements

2.3.1 Allergy sensitization – skin prick testing (SPT)

Allergic sensitization was assessed by standardized SPT to common inhalant and food allergens. Standardized SPT was conducted by trained doctors during the clinic visit at 18 months of age using 3 food allergens (cow's milk, peanut, and egg) and 3 house dust mites (Dermatophagoidespteronyssinus, Dermatophagoidesfarinae, Blomiatropicalis). Histamine and saline were used as positive and negative controls, respectively. Wheal size ≥3 mm was classified as positive. SPT was considered valid only if the positive wheal was ≥3 mm, and the negative control exhibited no wheal reaction. A positive SPT to at least one allergen was considered indicative of allergic sensitization.(96)

2.3.2 Early childhood rhinitis, eczema and wheezing

Information on clinical outcomes of potential allergic diseases(eczema, rhinitis and wheezing) was collected serially at 7 time points: 3 weeks, 3 months and every 3 months thereafter till 18 months of age. Trained interviewers administered standardized questionnaires adapted from the International Study of Asthma and Allergies Questionnaire (ISAAC)(97) to the mother or main caregiver. Rhinitis was defined as parents' positive response to the question: "At any time, has your child had running nose, blocked or congested nose, snoring or noisy breathing during sleep or when awake that has lasted for 2 or more weeks duration?" Doctor-diagnosed eczema was based on a positive answer to the question: "Has your child ever been diagnosed with eczema?" Wheezing was defined as "noisy breathing with a high-pitch, whistling sound heard from the chest, not the mouth". In order to decrease false positive reporting of wheezing, we additionally added another question in which nebulizer/inhaler usage by a doctor was assessed. Wheezing was diagnosed with positive responses to both questions: "Has your child ever wheezed?" and "Has your child been prescribed with nebulizer/inhaler treatment since the last visit?" After getting results from the questionnaires, phone calls were made to ask for further details. Presence of doctor diagnosed eczema, rhinitis, or wheezing was indicated by a positive response during any one of the 7 follow up questionnaires during the first 18 months of life.

2.3.3 Allergic diseases

Any allergic disease with positive SPT was defined as having any one of the above clinical outcomes (eczema, rhinitis, and wheezing) plus a positive

SPT.

2.4 Statistical methods

All statistics were performed by using the statistical software package IBM SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Two sample t-test was used for comparing means of continuous variables and chi-square test was used for comparing the distribution of categorical variables.Binary logistic regression models were used to test the independent associations between the various allergic outcomes (i.e. SPT, rhinitis, eczema, wheezing and any allergic disease with positive SPT in the offspring) and individual maternal PUFAs. PUFAs of interest were first treated as continuous explanatory variables (continuous model), and then categorized into quartiles within the total cohort to test for a possible non-linear relationship and to examine dose-response (categorical model).

In the models, we adjusted for maternal characteristics including maternal age, ethnicity, gravidity, education level, energy intake, and infant characteristics including gender, birth weight, gestational age, duration of breastfeeding, family history of allergic diseases, which includes allergic rhinitis, eczema and asthma in first degree relatives of the children(i.e. father, mother and/or sibling), exposure to environmental tobacco smoke, child day care attendance, and cat or dog at home during the period up to 18 months of age. Subgroup analysis was also performed in the group of children with no family history of allergic diseases to rule out the possibility of genetic susceptibility as a confounding factor.

To control for type 1 error due to the performance of multiple analyses, an adjusted P value < 0.01 (p=0.05 divided by 5 allergy outcomes) was used to indicate statistical significance. Results are presented as adjusted odds ratios (OR) with corresponding 95% confidence intervals.

Chapter 3. RESULTS

3.1 Maternal PUFA status and rates of allergy outcomes

3.1.1Maternal PUFA status

Of the 1162 women enrolled in the main GUSTO birth cohort, 998 mothers with singleton live births had blood samples available for measurement of plasma PCfatty acids. The median (range) percentages for total n-3 and n-6 PUFAs were 6.18% (2.22% - 13.97%) and 34.22% (10.77% - 51.29%), respectively. Median values with their 25th and 75th percentiles of the other fatty acids can be found in Table 3-1. Similar to previous findings, the predominant n-3 PUFA in maternal plasma was DHA, and the major n-6 PUFAs were LA and AA(19).

Table 3-1 Fatty acid composition of maternal plasma PC measured at 26-28 weeks of gestation.

Fatty acid exposure*	Median	25th and 75th percentiles
Total n-3 PUFAs %	6.18	5.00, 7.49
ALA %	0.19	0.10, 0.28
EPA %	0.52	0.35, 0.82
DPA %	0.55	0.46, 0.69
DHA %	4.61	3.60, 5.63
DHA+EPA %	5.24	4.14, 6.423
Total n-6 PUFAs %	34.22	32.38, 36.17
LA %	21.79	19.49, 24.00
AA %	7.80	6.80, 8.87
Total n-6:n-3 PUFAs	5.49	4.52, 6.94

PUFA, polyunsaturated fatty acid; ALA, a-linolenic acid, 18:3n-3;EPA, eicosapentaenoic acid, 20:5n-3; DPA, docosapentenoic acid, 22:5n-3; DHA,docosahexaenoic acid, 22:6n-3; LA, linoleic acid, 18:2n-6; AA, arachidonicacid, 20:4n-6.

N=998

*Fatty acids were expressed as percentage of total plasma fatty acids.

3.1.2 Rates of allergy outcomes

After excluding those with multiple missing confounders, only 960 mothers were included in the final analyses. Sample sizes varied for the individual outcomes due to different response rates(Figure3-1). SPT at 18 months was performed in 728 children, among which 103 (14.1%) showed a positive result. Of 808 children who had data on parental reported rhinitis up to 18 months of life, 214 (26.5%) had rhinitis. Of 833 children with data on parental reported doctor diagnosed eczema up to 18 months of life, 147 (17.6%) were diagnosed with eczema.Of 859 children with data on wheezing symptoms (parent-reported and use of nebulizer or inhaler), 94 (10.9%) had wheezing. Of 657 children with data on SPTand the occurrence of any allergic disease, 62 (9.4%) showed a positive result. Characteristics of mothers who agreed to have SPT performed on their children (n=728) and those who did not (n=232) were broadly similar(Table 3-2), except that those who agreed tended to be slightly older, and were more likely to have more than one child.

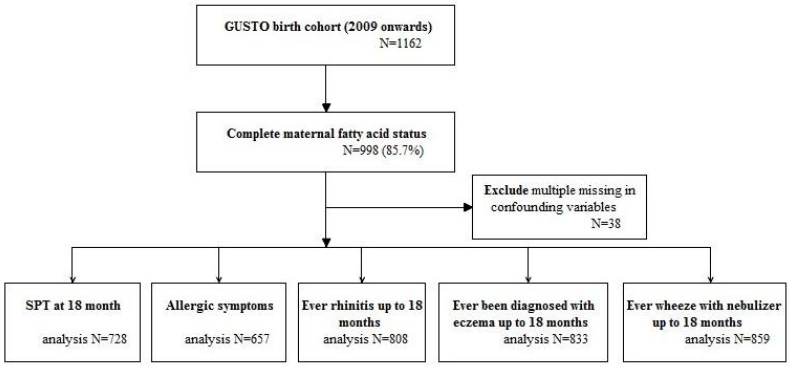

Figure3-1: Flow chart of the participantsin this study.

Table 3-2 Comparison of maternal characteristics of those with SPT data and those without SPT data.[1]

	With SPT		Without SPT		P-value*
	Mean	SD	Mean	SD	
n	728		232		
Maternalcharacteristics					
Age (years)	30.9	5.2	29.6	4.9	<0.005
Gravidity>1 (%)	60.6		48.5		<0.005
Educationalstatus (%)					0.63
Primary/Secondary	32.0		31.2		
Post-secondary	36.3		32.9		
University	33.5		35.9		
Ethnicity (%)					0.75
Chinese	54.3		56.7		
Malay	27.1		24.7		
Indian	18.6		18.6		
Totalenergyintake (KJ)	7891	2410	7698	2540	0.30
Totaln-3PUFAs†	6.4	1.9	6.2	1.8	0.17
Total n-6PUFAs†	34.2	3.3	34.1	3.7	0.79
n-6:n-3PUFAs ratio	5.8	2.0	6.0	2.1	0.26
Infantcharacteristics					
Gestationalage (weeks)	38.6	1.4	38.6	1.5	0.94
Male gender (%)	50.8		54.1		0.41
Monthsofbreastfeeding (%)					0.23
None	7.3		9.1		
<4	43.7		40.3		
≥4	41.3		32.0		
Unknown	7.7		18.6		
Birthweight (g)	3116	440	3056	471	0.07
Family history of allergic diseases (%)					0.66
No	57.3		13.9		
1 parent	29.1		8.2		
2 parents	8.4		2.2		
Sibling only	5.2		0.4		
Unknown	0		75.3		
Environmental smoking duringinfancy (%)					0.24
No	51.1		32.5		
Yes	32.1		26.0		
Unknown	16.8		41.6		
Childcare attendance during infancy (%)					0.87
No	75.7		51.9		
Yes	7.3		5.2		
Unknown	17.0		42.9		
Cat/dogathome during infancy (%)					0.82
No	74.3		51.9		
Yes	8.7		5.6		
Unknown	17.0		42.4		
Everrhinitis (%)	26.6		26.0		0.49
Evereczema (%)	17.1		20.3		0.22
Ever wheeze (%)	10.2		14.6		0.08

SPT, Skin prick testing; SD, standard deviation.

[1]Values reflect the mean and standard deviation for continuous variables or percentages (%) for categorical variables, unless otherwise specified.

[2]Fatty acids were expressed as percentage of total plasma fatty acids.

[3]P-values obtained by 2-sample T-test for continuous variables and chi-square tests for categorical variables; P values ≤ 0.05 is significant

3.2 Population characteristics

Table 3-3and Table 3-4 show the main characteristics of the study population and bivariate associations with the various clinical allergic outcomes. There was a higher tendency for infants with eczema, wheeze and any allergic disease with positive SPT to be breastfed for longer than 4 months. Prevalence of rhinitis and eczema was highest in infants with both parents having allergic disease compared to those with one parent having allergic disease and was lowest in those with no family history of allergic disease. There was a higher prevalence of rhinitis and wheeze seen in infants who attended childcare during infancy. Additionally, the prevalence of eczema was higher in children of first-time pregnancies and those whose mothers had higher educational qualifications, while the prevalence of wheeze was higher is male infants and in infants with shorter gestational age.For all of the clinical allergic outcomes, Malay infants have the highest prevalence, followed by Chinese infants with the lowest prevalence in the Indian infants. This coincided with the prevalence of infants having family history of allergic diseases among the ethnic groups. In addition, Chinese mothers tended to have the highest plasma PC n-3 PUFA levels, lowest plasma PC n-6 PUFA levels and n-6:n-3 PUFA ratio, while Malay mothers had the highest n-6:n-3 PUFA ratio (Table 3-5).

Table3-3Maternal characteristicsofthestudyparticipantsand bivariate associations with clinical allergic outcomes.

	Unit	SPT			any allergic diseases with SPT*			ever rhinitis			ever eczema			ever wheeze		
		no	yes	P-value†	no	yes	P-value†	No	yes	P-value†	no	yes	P-value†	no	yes	P-value†
		n=625	n=103		n=595	n=62		n=594	n=214		n=686	n=147		n=765	n=94	
Maternal characteristics																
Age	years	30.8(5.2)	31.5(5.3)	0.21	31(5.2)	32(5.2)	0.15	31.0(5.1)	30.6(5.6)	0.41	30.8(5.1)	31.3(5.2)	0.25	30.8(5.1)	30.1(5.3)	0.22
Gravidity >1	No	248(86.4)	39(13.6)	0.75	239(91.9)	21(8.1)	0.41	249(72.8)	93(27.2)	0.70	278(79.2)	73(20.8)	0.04	328(91.1)	32(8.9)	0.12
	Yes	377(85.5)	64(14.5)		356(89.7)	41(10.3)		345(74)	121(26)		408(84.6)	74(15.4)		437(87.6)	62(12.4)	
Educational status	Primary/Secondary	189(85.9)	31(14.1)	0.68	176(91.2)	17(8.8)	0.75	170(73.3)	62(26.7)	0.99	211(86.8)	32(13.2)	0.01	226(90.8)	23(9.2)	0.40
	Post-secondary	230(87.1)	34(12.9)		217(91.2)	21(8.8)		208(73.5)	75(26.5)		250(83.6)	49(16.4)		267(87.3)	39(12.7)	
	University	206(84.4)	38(15.6)		202(89.4)	24(10.6)		216(73.7)	77(26.3)		225(77.3)	66(22.7)		272(89.5)	32(10.5)	
Ethnicity	Chinese	340(86.1)	55(13.9)	0.05	333(92)	29(8)	0.01	348(76)	110(24)	<0.005	375(80.6)	90(19.4)	0.02	432(89.6)	50(10.4)	<0.005
	Malay	161(81.7)	36(18.3)		150(85.2)	26(14.8)		128(64.3)	71(35.7)		178(80.5)	43(19.5)		182(83.1)	37(16.9)	
	Indian	124(91.2)	12(8.8)		112(94.1)	7(5.9)		118(78.1)	33(21.9)		133(90.5)	14(9.5)		151(95.6)	7(4.4)	
Total energy intake	KJ	7845(2414)	8171(2380)	0.20	7928 (2469)	8247(2117)	0.33	7858(2469)	8021(2381)	0.40	7849(2481)	8176(2222)	0.14	7853(2423)	7858(2481)	0.99
Total n-3PUFAs‡	%	6.4(1.9)	6.5(1.8)	0.50	6.4(1.9)	6.8(1.8)	0.21	6.4(1.9)	6.6(1.8)	0.15	6.4(1.9)	6.5(1.9)	0.50	6.4(1.9)	6.6(2.0)	0.22
Total n-6PUFAs‡	%	34.3(3.3)	33.8(3.5)	0.18	34.2(3.4)	34(2.8)	0.66	34.2(3.5)	34.1(2.9)	0.87	34.1(3.5)	34.1(2.9)	0.99	34.3(3.4)	33.4(2.9)	0.02
n-6:n-3PUFAs ratio		5.9(2.0)	5.6(1.9)	0.28	5.8(2.0)	5.5(1.9)	0.20	5.9(2.1)	5.6(1.6)	0.01	5.8(2.0)	5.7(1.9)	0.57	5.9(2.0)	5.5(1.8)	0.11

SD, standard deviation; SPT, Skin prick testing.

Values reflect the mean (standard deviation) for continuous variables orabsolute numbers (percentage) for categorical variables, unless otherwise specified.

[1]Any allergic diseases with SPT was defined as having any one of the clinical outcomes with a positive SPT.

[2]P-valuesobtainedby 2 sample t-testforcontinuousvariablesandchi-squaretestsforcategoricalvariables; $P \leq 0.05$ is significant.

[3]Fatty acids were expressed as percentage of total plasma fatty acids.

708 Table3-4Infant characteristicsand bivariate associations with clinical allergic outcomes.

	Unit	SPT			any allergic diseases with SPT*			ever rhinitis			ever eczema			ever wheeze		
		no	yes	P-value†	no	yes	P-value†	No	yes	P-value†	no	yes	P-value†	no	yes	P-value†
		n=625	n=103		n=595	n=62		n=594	n=214		n=686	n=147		n=765	n=94	
Infant characteristics																
Gestational age	weeks	38.6(1.39)	38.8(1.41)	0.41	38.7(1.35)	38.6(1.56)	0.51	38.7(1.38)	38.6(1.21)	0.30	38.7(1.32)	38.7(1.76)	0.91	38.8(1.3)	38.3(1.66)	<0.005
Gender	Male	310(83.8)	60(16.2)	0.11	297(89.5)	35(10.5)	0.35	296(71.2)	120(28.8)	0.13	346(80.7)	83(19.3)	0.20	389(87)	58(13)	0.05
	Female	315(88)	43(12)		298(91.7)	27(8.3)		298(76)	94(24)		340(84.2)	64(15.8)		376(91.3)	36(8.7)	
Months of breastfeeding	none	47(88.7)	6(11.3)	0.46	46(95.8)	2(4.2)	0.06	52(77.6)	15(22.4)	0.70	57(86.4)	9(13.6)	0.02	67(95.7)	3(4.3)	0.06
	＜4	279(87.7)	39(12.3)		266(93)	20(7)		255(74.1)	89(25.9)		309(86.6)	48(13.4)		333(90)	37(10)	
	≥4	252(83.7)	49(16.3)		245(88.1)	33(11.9)		250(72.9)	93(27.1)		273(78.2)	76(21.8)		323(88)	44(12)	
	unknown	47(83.9)	9(16.1)		38(84.4)	7(15.6)		37(68.5)	17(31.5)		47(77)	14(23)		42(80.8)	10(19.2)	
Birth weight	g	3107(444)	3176(413)	0.14	3118(434)	3157(414)	0.66	3117(447)	3135(417)	0.61	3100(433)	3159(468)	0.14	3125(446)	3061(407)	0.19
Family history of allergic diseases	No	361(86.6)	56(13.4)	0.75	334(92.5)	27(7.5)	0.27	318(80.1)	79(19.9)	<0.005	370(87.1)	55(12.9)	<0.005	399(91.3)	38(8.7)	0.11
	1 parent	178(84.0)	34(16.0)		173(87.4)	25(12.6)		143(66.8)	71(33.2)		169(75.8)	54(24.2)		198(88.8)	25(11.2)	
	2 parents	52(85.2)	9(14.8)		50(87.7)	7(12.3)		35(55.6)	28(44.0)		47(74.6)	16(25.4)		53(82.8)	11(17.2)	
	Sibling only	34(89.5)	4(10.5)		32(91.4)	3(8.6)		25(67.6)	12(32.4)		31(81.6)	7(18.4)		32(82.1)	7(17.9)	
	Unknown							73(75.3)	24(24.7)		69(82.1)	15(17.9)		83(86.5)	13(13.5)	
Environmental smoking during infancy	No	312(83.9)	60(16.1)	0.23	309(89.6)	36(10.4)	0.36	307(75.1)	102(24.9)	0.54	339(81.7)	76(18.3)	0.82	391(90.1)	43(9.9)	0.35
	Yes	208(88.9)	26(11.1)		197(92.9)	15(7.1)		184(72.7)	69(27.3)		226(82.5)	48(17.5)		244(86.8)	37(13.2)	
	unknown	105(86.1)	17(13.9)		89(89)	11(11)		103(70.5)	43(29.5)		121(84)	23(16)		130(90.3)	14(9.7)	
Childcare attendance during infancy	No	472(85.7)	79(14.3)	0.96	460(91.1)	45(8.9)	0.68	453(76.1)	142(23.9)	<0.005	516(82.8)	107(17.2)	0.18	586(90.6)	61(9.4)	<0.005
	Yes	46(86.8)	7(13.2)		44(88)	6(12)		35(55.6)	28(44.4)		45(73.8)	16(26.2)		44(69.8)	19(30.2)	
	unknown	107(86.3)	17(13.7)		91(89.2)	11(10.8)		106(70.7)	44(29.3)		125(83.9)	24(16.1)		135(90.6)	14(9.4)	
Cat/dog at home during infancy	No	464(85.8)	77(14.2)	0.99	456(91.4)	43(8.6)	0.34	439(74.3)	152(25.7)	0.67	506(82.5)	107(17.5)	0.33	566(89)	70(11)	0.91
	Yes	54(85.7)	9(14.3)		48(85.7)	8(14.3)		49(73.1)	18(26.9)		55(76.4)	17(23.6)		66(88)	9(12)	
	unknown	107(86.3)	17(13.7)		91(89.2)	11(10.8)		106(70.7)	44(29.3)		125(84.5)	23(15.5)		133(89.9)	15(10.1)	

SD, standard deviation; SPT, Skin prick testing;

Values reflect the mean (standard deviation) for continuous variables or absolute numbers (percentage) for categorical variables, unless otherwise specified.;

[1]Any allergic diseases with SPT was defined as having any one of the clinical outcomes with a positive SPT.;

[2]P-values obtained by2 sample t-test for continuous variables and chi-square tests for categorical variables; $P \leqslant 0.05$ is significant.

Table3-5Comparison of maternal plasma PC PUFAs and family history of allergic diseases across ethnicities.

	Chinese		Malay		Indian		P-value*
	Mean	SD	Mean	SD	Mean	SD	
n	527		254		179		
Total n-3PUFAs†	6.7	1.9	6.0	1.7	6.0	1.8	<0.005
Total n-6PUFAs†	33.8	3.4	34.1	3.2	35.3	3.5	<0.005
n-6:n-3PUFAs ratio	5.5	1.8	6.1	1.9	6.5	2.4	<0.005
Family history of allergic diseases (%)							0.18
No	46.2		44.9		51.4		
1 parent	25.7		26.4		16.2		
2 parents	6.1		7.1		8.9		
Sibling only	4.0		3.5		5.0		
Unknown	18.1		18.1		18.4		

SD, standard deviation.

Values reflect the mean and standard deviation for continuous variables or percentages (%) for categorical variables, unless otherwise specified.

[1]P-values obtained by one-way analysis of variance (ANOVA) for continuous variables and chi-square tests for categorical variables; P values ≤ 0.05 is significant.

[2]Fatty acids were expressed as percentage of total plasma fatty acids.

3.3 Association between maternal PUFA status and offspring allergy outcomes

In bivariate analyses using quartiles of PUFAs(Table 3-6), weak trends ofhigher maternal plasma PC n-3 PUFAs being associated with any allergic diseases with positive SPT in infants (P=0.07),lower maternal plasma PC n-6 PUFAs being associated with wheeze in infants (P=0.06) and lower maternal n-6:n-3 PUFA ratio being associated with wheeze and any allergic diseases with positive SPT in infants (P=0.06;P=0.07) were observed. These trends were not as clearly observed in the group of infants without family history of allergic diseases (P >0.1 for all)(Tables 3-7).

733 Table 3-6Infant allergy outcomesaccordingtoquartilesofmaternaltotalplasma
734 PC n-3 PUFA, n-6 PUFA status and n-6:n-3 PUFA ratio.

	Q1	**Q2**	**Q3**	**Q4**	**P-value**[1]
Totaln-3 PUFAs[2]	2.2-5.0	5.1-6.1	6.2-7.5	7.6-14.0	
n	240	240	240	240	
Allergy outcomes					
SPT (%)	13.2	13.1	11.7	18.3	0.22
Everrhinitis (%)	20.7	28.6	30.2	25.9	0.25
Evereczema (%)	17.6	18.5	14.5	19.9	0.79
Everwheeze (%)	10.2	10.0	11.3	12.2	0.43
Any allergicdiseases with SPT[3] (%)	6.6	9.4	7.8	13.3	0.07
	Q1	**Q2**	**Q3**	**Q4**	**P-value***
Totaln-6 PUFAs[2]	10.8-32.3	32.4-34.2	34.3-36.1	36.2-51.3	
n	240	240	240	240	
Allergy outcomes					
SPT (%)	17.5	9.9	13.2	16.0	0.92
Everrhinitis (%)	27.1	24.9	29.6	24.5	0.45
Evereczema (%)	15.7	18.3	17.6	18.9	0.45
Everwheeze (%)	13.6	11.6	11.0	7.6	0.06
Any allergicdiseases with SPT[3] (%)	9.6	7.9	11.7	8.7	0.93
	Q1	**Q2**	**Q3**	**Q4**	**P-value***
n-6:n-3 PUFAs ratio	1.9-4.5	4.6-5.4	5.5-6.9	7.0-16.6	
n	240	240	240	240	
Allergy outcomes					
SPT (%)	18.8	11.5	13.4	12.5	0.13
Everrhinitis (%)	26.6	27.9	32.0	18.9	0.21
Evereczema (%)	20.6	11.4	19.7	18.8	0.88
Everwheeze (%)	14.3	10.6	10.0	8.5	0.06
Any allergicdiseases with SPT[3](%)	12.6	8.8	9.4	6.4	0.07

735 Q,quartile; SD, standard deviation; SPT, Skin prick testing.
736 Values reflect the percentages (%) for categorical variables, unless otherwise
737 specified.
738 [1]P-valuesobtainedbychi-squaretestsforcategoricalvariables.
739 [2]Fattyacidswereexpressedaspercentageoftotalplasmafattyacids.
740 [3]Any allergicdiseases with SPT was defined as having any one of the clinical
741 outcomes with a positive SPT

Table 3-7Infant allergy outcomesaccordingtoquartilesofmaternaltotalplasma PC n-3 PUFA, n-6 PUFA status and n-6:n-3 PUFA ratioin the group without family history of allergic diseases.

	Q1	**Q2**	**Q3**	**Q4**	**P-value**[1]
Total n-3 PUFAs[2]	2.2-5.0	5.1-6.1	6.2-7.5	7.6-14.0	
n	115	115	115	115	
Allergy outcomes					
SPT (%)	11.8	12.0	12.4	17.5	0.25
Ever rhinitis (%)	11.6	23.1	26.0	19.4	0.17
Ever eczema (%)	9.2	13.6	13.1	16.2	0.17
Ever wheeze (%)	8.7	6.2	9.2	10.7	0.43
Any allergic diseases with SPT[3] (%)	4.8	8.5	5.6	10.6	0.24
	Q1	**Q2**	**Q3**	**Q4**	**P-value***
Total n-6 PUFAs[2]	10.8-32.3	32.4-34.2	34.3-36.1	36.2-51.3	
n	115	115	115	115	
Allergy outcomes					
SPT (%)	18.4	9.3	13.1	12.9	0.39
Ever rhinitis (%)	18.6	17.8	23.9	20.4	0.54
Ever eczema (%)	11.4	14.5	13.3	13.2	0.78
Ever wheeze (%)	10.9	9.6	9.3	4.7	0.12
Any allergic diseases with SPT[3] (%)	10.0	5.5	9.4	5.2	0.38
	Q1	**Q2**	**Q3**	**Q4**	**P-value***
n-6:n-3 PUFAs ratio[2]	1.9-4.4	4.5-5.4	5.5-6.9	7.0-15.8	
n	115	115	115	115	
Allergy outcomes					
SPT (%)	16.7	13.0	10.6	13.5	0.43
Ever rhinitis (%)	21.2	22.0	22.9	14.3	0.28
Ever eczema (%)	16.4	12.8	13.1	10.0	0.20
Ever wheeze (%)	10.8	9.8	7.3	6.7	0.22
Any allergic diseases with SPT[3] (%)	8.5	9.6	4.7	6.8	0.42

Q, quartile; SD, standard deviation; SPT, Skin prick testing.

Values reflect the percentages (%) for categorical variables, unless otherwise specified.

[1]P-values obtained by chi-square tests for categorical variables.

[2]Fatty acids were expressed as percentage of total plasma fatty acids.

[3]Any allergic diseases with SPT was defined as having any one of the clinical outcomes plus a positive SPT.

Upon adjustment for potential confounders (Table 3-8), no statistically significant linear relationships between the individual maternal PUFAs as continuous variables and any of the various allergic outcomes were observed. From quartile analyses, a weak positive trend between maternal plasma PC n-3 PUFAs and any allergic diseases with positive SPT in infants persisted.The odds ratio of any allergic diseases with positive SPT was highest (OR=2.09) in the highest quartile of n-3 PUFAs when compared to the lowest quartile (reference), although this was not statistically significant. This was also observed in the group of infants without family history of allergic diseases (Table 3-9).No clear associations were observed with maternal plasma PC total n-6 PUFA statusand the risk of having any allergic disease with SPT up to 18 months of age. Correspondingly, a negative trend was observed between maternal plasma PC n-6: n-3 PUFA ratio and the risk of having any allergic disease with SPT up to 18 months of age in the whole cohort only, although this did not reach statistical significance.

While the odd ratios for wheezing in infants appear to be lower with increasing quartiles of maternal plasma PC n-6 PUFAs and n-6:n-3 PUFA ratios, and the odds ratio for ever eczema in infants appear to be higher with increasing quartiles of maternal plasma PC n-6 PUFAs in both the whole cohort and in the group of infants without family history of allergic diseases, these associations were not statistically significant. The odd ratios for ever rhinitis in infants appear to be higher with increasing quartiles of maternal plasma PC n-3 PUFAs and n-6:n-3 PUFA ratios, but only up to the 3rd quartile, in both the whole cohort and in the group of infants without family history of allergic diseases(Table 3-8 and Table 3-9). However, these associations were not statistically significant.

Table 3-8. Association between maternal plasma PC PUFA status at 26-28 weeks of pregnancy and early childhood allergic diseases

		Adjusted OR (95% CI)[1]				
Quartiles of plasma fatty acids	Range(wt%)	SPT n=728[3,4]	Any allergic diseases with SPT[2] n=657[3,4]	Ever rhinitis n=808[3,4]	Ever eczema n=833[3,4]	Ever wheeze n=859[3,4]
Total n-3 PUFAs						
Continuous model		1.03 (0.91,1.16)	1.08 (0.93,1.25)	1.07 (0.98,1.17)	0.99 (0.89,1.09)	1.06 (0.93,1.20)
Categorical model						
1	≤5.00	1(reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	5.01-6.18	0.91(0.48,1.72)	1.38 (0.58,3.28)	1.56 (0.96,2.54)	1.02 (0.60,1.74)	0.90 (0.45,1.78)
3	6.19-7.49	0.83(0.43,1.58)	1.10 (0.45,2.71)	1.67 (1.03,2.70)	0.67 (0.38,1.18)	1.09 (0.56,2.13)
4	≥7.50	1.41(0.77,2.58)	2.09 (0.91,4.78)	1.34 (0.81,2.21)	0.93 (0.55,1.60)	1.12 (0.57,2.2)
Total n-6 PUFAs						
Continuous model		0.97 (0.90,1.03)	1.00 (0.92,1.09)	1.01 (0.96,1.06)	1.03 (0.98,1.10)	0.94 (0.88,1.01)
Categorical model						
1	≤32.38	1 (reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	32.39-34.22	0.48 (0.25,0.90)	0.75 (0.34,1.68)	0.92 (0.58,1.46)	1.29 (0.76,2.20)	0.79 (0.43,1.46)
3	34.23-36.17	0.71 (0.39,1.28)	1.26 (0.59,2.65)	1.17 (0.74,1.84)	1.37 (0.80,2.35)	0.76 (0.40,1.43)
4	≥36.18	0.98 (0.55,1.74)	1.02 (0.47,2.23)	1.00 (0.63,1.60)	1.56 (0.91,2.68)	0.67 (0.34,1.34)
n-6:n-3 PUFAsratio						
Continuous model		0.96 (0.85,1.08)	0.93 (0.79,1.09)	0.91 (0.83,1.00)	1.03 (0.93,1.14)	0.93 (0.82,1.07)
Categorical model						
1	≤4.52	1 (reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	4.53-5.49	0.60 (0.33,1.10)	0.70 (0.34,1.46)	1.11 (0.7,1.75)	0.55 (0.32,0.97)	0.70 (0.38,1.30)
3	5.50-6.94	0.67 (0.37,1.21)	0.75 (0.36,1.56)	1.34 (0.85,2.11)	1.19 (0.72,1.97)	0.64 (0.34,1.23)
4	≥6.95	0.66 (0.36,1.22)	0.52 (0.23,1.21)	0.66 (0.40,1.10)	1.21 (0.71,2.06)	0.63 (0.32,1.25)

OR, odds ratio; SPT, skin prick testing; PUFA, polyunsaturated fatty acid.

[1]Odds ratios (ORs) for the independent association between maternal total n-3, total n-6 PUFA status and n-6 to n-3 PUFAs ratio in plasma phosphatidylcholine at 26-28 weeks of pregnancy and various childhood allergic outcomes. Binary logistic regressions were performed using PUFAs as continuous variables (continuous model) and then as categorical variables (divided into quartiles in categorical model) respectively.

[2]Any allergic diseases with SPT was defined as having any one of the clinical outcomes plus a positive SPT.

[3]Number of cases: SPT at 18 months of age 103/728, any allergic diseases with SPT 62/657, ever rhinitis 0 to 18 months of age 214/808, ever diagnosed eczema 147/833, and ever wheezing with nebulizer 94/859.

[4]Adjusted for maternal age, education level, energy intake, infant ethnicity, gender, gravidity, birth weight, gestational age, length of breastfeeding, family history of allergic diseases, exposure to environmental tobacco smoke, child day care attendance, cat/dog at home during infancy.

Table 3-9. Association between maternal plasma PC PUFA status at 26-28 weeks of pregnancy and early childhood allergic diseases in the group with no family history of allergic diseases

		Adjusted OR (95% CI)[1]				
Quartiles of plasma fatty acids	Range(wt%)	SPT N=418[34]	Any allergic diseases with SPT[2] N=362[34]	Ever rhinitis N=398[34]	Ever eczema N=426[34]	Ever wheeze N=438[34]
Total n-3 PUFAs						
Continuous model		1.02 (0.87,1.20)	1.07 (0.86,1.33)	1.08 (0.95,1.24)	1.07 (0.92,1.25)	1.04 (0.86,1.27)
Categorical model						
1	≤5.08	1(reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	5.09-6.21	0.94 (0.40,2.23)	1.49 (0.39,5.62)	2.54 (1.12,5.77)	1.40 (0.56,3.49)	0.64 (0.21,1.92)
3	6.22-7.63	1.00 (0.41,2.40)	0.92 (0.22,3.92)	2.47 (1.07,5.68)	1.27 (0.50,3.21)	0.71 (0.25,2.05)
4	≥7.64	1.36 (0.59,3.14)	1.84 (0.50,6.84)	1.99 (0.85,4.67)	1.43 (0.58,3.54)	1.10 (0.40,3.05)
Total n-6 PUFAs						
Continuous model		0.95 (0.87,1.04)	0.98 (0.86,1.11)	1.04 (0.96,1.12)	1.05 (0.96,1.14)	0.96 (0.86,1.08)
Categorical model						
1	≤32.34	1 (reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	32.35-34.32	0.43 (0.18,1.00)	0.50 (0.15,1.66)	0.99 (0.46,2.11)	1.39 (0.60,3.21)	0.69 (0.27,1.78)
3	34.33-36.19	0.69 (0.32,1.51)	1.11 (0.38,3.26)	1.34 (0.64,2.81)	1.48 (0.63,3.48)	0.83 (0.32,2.15)
4	≥36.20	0.75 (0.34,1.67)	0.64 (0.19,2.16)	1.38 (0.66,2.89)	1.50 (0.63,3.57)	0.49 (0.16,1.58)
n-6:n-3 PUFAs ratio						
Continuous model		0.94 (0.80,1.11)	0.91 (0.71,1.16)	0.91 (0.79,1.04)	0.96 (0.81,1.12)	0.97 (0.79,1.20)
Categorical model						
1	≤4.45	1 (reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	4.46-5.48	0.90 (0.41,1.99)	1.38 (0.48,4.01)	1.02 (0.49,2.10)	0.90 (0.40,1.99)	0.70 (0.27,1.84)
3	5.49-6.92	0.64 (0.28,1.50)	0.57 (0.16,2.11)	1.22 (0.60,2.47)	0.93 (0.42,2.05)	0.68 (0.24,1.89)
4	≥6.93	1.05 (0.46,2.41)	1.16 (0.34,3.92)	0.64 (0.29,1.42)	0.83 (0.34,2.04)	0.69 (0.23,2.05)

OR, odds ratio; SPT, skin prick testing; PUFA, polyunsaturated fatty acid.

[1]Odds ratios (ORs) for the association between maternal total n-3and total n-6 PUFA status and n-6 to n-3 PUFAs ratio in plasma phosphatidylcholine at 26-28 weeks of pregnancy and various childhood allergic outcomes, respectively. Binary logistic regressions were performed using PUFAs as continuous variables (continuous model) and then as categorical variables (divided into quartiles in categorical model) respectively.

[2]Any allergic diseases with SPT was defined as having any one of the clinical outcomes plus a positive SPT.

[3]Number of cases: SPT at 18 months of age 56/418, any allergic diseases with SPT27/362, ever rhinitis 0 to 18 months of age 75/398, ever diagnosed eczema 56/426, and ever wheezing with nebulizer 38/438.

[4]Adjusted for maternal age, education level, energy intake, infant ethnicity, gender, gravidity, birth weight, gestational age, length of breastfeeding,exposure to environmental tobacco smoke, child day care attendance, cat/dog at home during infancy.

When examining the individual PUFAs (ALA, EPA, DPA, DHA, EPA+DHA, LA, and AA), it appears that DPA and EPA were the key n-3 PUFAs driving the association with higher risk of any allergic diseases with positive SPT, while DHA was the key n-3 PUFA driving the association with higher risk of rhinitis. For the two n-6 PUFAs examined (LA, AA), there was no clear association with higher risk of wheeze and eczema (Table 3-10). Analyses were also conducted using PUFA concentrations, rather than percentages, with allergic outcomes and results were not different to those described above (data not shown).

Table 3-10. Association between specific maternal plasma PC PUFAs at 26-28 weeks of pregnancy and early childhood allergic diseases

		Adjusted OR (95%CI) *				
Quartiles of plasma fatty acids	Range(wt%)	SPT N=728‡§	Any allergic diseases with SPT† N=657‡§	Ever rhinitis N=808‡§	Ever eczema N=833‡§	Ever wheeze N=859‡§
n-3 PUFAs						
ALA						
Continuous model		0.85 (0.18,4.01)	0.79 (0.11,5.78)	0.47 (0.14,1.63)	1.26 (0.34,4.62)	0.59 (0.10,3.42)
Categorical model						
1	≤0.10	1 (reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	0.11-0.18	0.80 (0.43,1.49)	1.10 (0.49,2.47)	1.18 (0.74,1.89)	0.87 (0.50,1.51)	1.22 (0.61,2.44)
3	0.19-0.27	0.92 (0.51,1.68)	1.34 (0.61,2.92)	0.95 (0.59,1.52)	1.07 (0.64,1.81)	1.77 (0.91,3.46)
4	≥0.28	0.85 (0.46,1.55)	0.99 (0.44,2.19)	0.86 (0.54,1.38)	0.87 (0.51,1.47)	1.12 (0.56,2.24)
EPA						
Continuous model		0.97 (0.66,1.44)	1.05 (0.66,1.68)	1.03 (0.78,1.36)	1.03 (0.74,1.41)	1.08 (0.75,1.57)
Categorical model						
1	≤0.35	1 (reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	0.36-0.51	1.12 (0.57,2.21)	1.07 (0.42,2.74)	1.09 (0.68,1.77)	1.14 (0.64,2.04)	1.07 (0.52,2.21)
3	0.52-0.82	1.26 (0.65,2.47)	1.93 (0.80,4.67)	1.02 (0.63,1.66)	1.07 (0.60,1.89)	1.19 (0.60,2.36)
4	≥0.83	1.82 (0.94,3.50)	1.83 (0.76,4.45)	1.04 (0.64,1.69)	1.20 (0.68,2.13)	1.20 (0.60,2.39)
DPA						
Continuous model		1.44 (0.49,4.25)	2.99 (0.79,11.39)	1.10 (0.47,2.55)	1.79 (0.70,4.60)	1.21 (0.37,3.95)
Categorical model						
1	≤0.46	1 (reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	0.47-0.55	0.93 (0.48,1.80)	1.18 (0.48,2.90)	0.72 (0.45,1.17)	1.74 (0.97,3.11)	0.60 (0.31,1.17)
3	0.56-0.69	1.20 (0.62,2.29)	1.38 (0.57,3.35)	0.80 (0.49,1.28)	1.62 (0.90,2.92)	0.64 (0.33,1.26)
4	≥0.70	1.46 (0.78,2.72)	2.05 (0.91,4.61)	1.03 (0.65,1.64)	1.54 (0.86,2.76)	0.68 (0.36,1.30)
DHA						
Continuous model		1.03 (0.89,1.20)	1.11 (0.92,1.35)	1.13 (1.00,1.27)	0.96 (0.84,1.09)	1.07 (0.91,1.27)
Categorical model						
1	≤3.60	1 (reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	3.16-4.59	0.90 (0.48,1.65)	0.92 (0.40,2.09)	1.53 (0.94,2.50)	1.02 (0.61,1.70)	0.95 (0.49,1.86)
3	4.60-5.63	0.73 (0.38,1.40)	0.83 (0.35,1.98)	1.97 (1.22,3.21)	0.61 (0.35,1.07)	0.98 (0.49,1.96)

4	≥5.64	1.24 (0.69,2.24)	1.51 (0.70,3.26)	1.42 (0.87,2.32)	0.80 (0.47,1.35)	1.15 (0.60,2.22)
DHA+EPA						
Continuous model		1.02 (0.90,1.16)	1.08 (0.92,1.27)	1.09 (0.99,1.21)	0.97 (0.87,1.09)	1.06 (0.93,1.22)
Categorical model						
1	≤4.14	1 (reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	4.15-5.21	0.83 (0.44,1.57)	1.06 (0.45,2.50)	1.71 (1.05,2.79)	1.20 (0.70,2.03)	1.25 (0.64,2.44)
3	5.22-6.42	0.85 (0.45,1.61)	1.23 (0.52,2.91)	1.72 (1.05,2.80)	0.77 (0.44,1.34)	0.97 (0.48,1.95)
4	≥6.43	1.20 (0.66,2.19)	1.60 (0.71,3.61)	1.40 (0.85,2.31)	0.85 (0.49,1.47)	1.20 (0.61,2.36)
n-6 PUFAs						
LA						
Continuous model		0.97 (0.91,1.03)	1.00 (0.92,1.09)	1.00 (0.95,1.05)	1.02 (0.97,1.08)	0.95 (0.88,1.02)
Categorical model						
1	≤19.49	1 (reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	19.50-21.79	1.03 (0.57,1.86)	1.38 (0.64,2.97)	1.31 (0.83,2.07)	1.17 (0.68,2.01)	0.54 (0.28,1.05)
3	21.80-24.00	0.70 (0.37,1.30)	0.70 (0.30,1.65)	0.91 (0.56,1.46)	1.09 (0.63,1.87)	0.89 (0.48,1.62)
4	≥24.01	0.95 (0.52,1.72)	1.18 (0.55,2.54)	1.07 (0.67,1.72)	1.41 (0.83,2.40)	0.62 (0.32,1.20)
AA						
Continuous model		0.99 (0.86,1.13)	1.01 (0.85,1.19)	1.04 (0.94,1.15)	0.94 (0.83,1.07)	0.99 (0.86,1.15)
Categorical model						
1	≤6.80	1 (reference）	1 (reference）	1 (reference）	1 (reference）	1 (reference）
2	6.81-7.79	1.34 (0.74,2.42)	1.43 (0.68,3.04)	0.86 (0.54,1.36)	1.07 (0.64,1.78)	0.93 (0.50,1.73)
3	7.80-8.87	1.16 (0.62,2.17)	1.52 (0.68,3.40)	1.18 (0.74,1.87)	1.40 (0.84,2.34)	0.83 (0.42,1.63)
4	≥8.88	1.22 (0.63,2.35)	1.09 (0.45,2.61)	0.98 (0.60,1.60)	0.66 (0.36,1.23)	1.06 (0.54,2.08)

OR, odds ratio; SPT, skin prick testing.ALA, a-linolenic acid, 18:3n-3; EPA, eicosapentaenoic acid, 20:5n-3; DPA, docosapentaenoic acid, 22:5n-3; DHA, docosahexaenoic acid, 22:6n-3; LA, linoleic acid, 18:2n-6; AA, arachidonic acid, 20:4n-6.

*Odds ratios (ORs) for the association between maternal total n-3and total n-6 PUFA status and n-6 to n-3 PUFAs ratio in plasma phosphatidylcholine at 26-28 weeks of pregnancy and various childhood allergic outcomes, respectively. Binary logistic regressions were performed using PUFAs as continuous variables (continuous model) and then as categorical variables (divided into quartiles in categorical model) respectively.

†Any allergic diseases with SPTwas defined as having any one of the clinical outcomes plus a positive SPT.

‡Number of cases: SPT at 18 months of age 103/728, any allergic diseases with SPT 62/657, ever rhinitis 0 to 18 months of age 214/808, ever diagnosed eczema 147/833, and ever wheezing with nebulizer 94/859.

§Adjusted for maternal age, education level, energy intake, infant ethnicity, gender, gravidity, birth weight, gestational age, length of breastfeeding, family history of allergic diseases, exposure to environmental tobacco smoke, child day care attendance, cat/dog at home during infancy.

Chapter 4. DISCUSSION

In this Asian birth cohort study, we did not find any significant protective effects of higher percentages of n-3 PUFAs or lower percentages of n-6 PUFAs in maternalplasmaPCagainst offspring allergic diseases in early childhood.

These results are in line with the large ALSPAC cohort(19)that showed no significant relation between maternal red cell PUFAs and offspring wheezing and eczema before 4 years of age, and a small study by Yu and Bjorksten(20)that found no significantassociation between maternal serum PUFAs and offspring asthma, eczema, allergic rhinoconjunctivitisand SPTupto 6 years of ageamong 47 mother-child pairs.The levels of n-3 PUFAs in the above two studies appear to be lower compared to this study (DHA+EPA median level for ALSPACstudy =2.62%; meanlevel in Yu*et al.*'s study=2.72%). This most likely reflects the different fractions reported which have different PUFA contents.Despite lower levels of maternalplasma PC total n-3 PUFAs(median=5.01%) than in the current study,the SWS study(17)reported a modest protective effect of DHA, EPA and total n-3 PUFAs against non-atopic persistent wheezing up to 6 years of age, but not on other phenotypes of wheezing. In contrast, we found a weak trend of higher total n-6 PUFAs and lower likelihood of ever wheeze in our cohort. A possible explanation for the difference in our results could be the specific wheezing patterns that SWS used, which were not captured in our study. Another possible explanation is the younger age of offspring in our study group, as respiratory allergy usually occurs at an older age (from preschool age)(28). Interestingly, the KOALA Birth Cohort(18)unexpectedly reported a protective effect of maternalAA

against eczema in the first 7 months of life, and ofthe ratio of n-6 to n-3 PUFAs against eczema in 6-7 yearold children. This is against the widely held notion that excessive AA and a high ratio of n-6 to n-3 PUFAs might increase the risk of allergic disease(5, 69). In contrast, we found a weak trend of increased total n-6 PUFAs and increased likelihood of ever eczema.

The inconsistent results emerging from the above observational studies are in contrast to the results from some interventional studies using fish oil supplementation.Fish oil supplementation during late pregnancy appears to protect against developing a positive SPT, food allergy and IgE-associated eczema and asthma in the offspring(12-14). The EPA+DHA levels in plasma phospholipids achieved in the fish oil supplement groups of these randomized trials(86) were higher (mean= 8.02%) than the levels in our cohort (mean=5.37%) and in other cohorts (ALSPAC median in red blood cells, 2.62%)(19). Therefore, it is possible that the protective effect of n-3 fatty acids can only be observed with the high intake achieved by supplementation, rather than the lower intakes consumed by the general population.

Another possible explanation for the lack of association in our study is that children of age 18 months may be too young for allergic evaluation, as many symptoms of wheezing, rhinitis and eczema are yet not associated with obvious allergy (i.e. positive SPT)(28). Further follow-up is necessary, as although the prevalence of allergic diseases increases with age, it has not been elucidated whether maternal PUFA status during pregnancy has a long-term effect and influences allergy development in children beyond the age of 18 months.

The present study has some methodological strengths. Recall bias of the allergic clinical outcomes was reduced by the repeated questionnaires with relatively short time-intervals and phone call confirmation after interviews, and data on confounding variables were collected prospectively.Blood samples were used to measure PC PUFA concentrations, which would be a more reliable nutrient biomarker than dietary recalls of PUFA intakes, which can be subjected to recall bias and under-reporting.

Some limitations in the current study merit consideration. Firstly, maternal plasma PC PUFA levels in our analysis were based on a single measurement at 26-28 weeks of pregnancy, which only reflects recent fatty acid intake in the proceeding few weeks, rather than long-term intake(71, 98-100). Therefore, it may not reflect levels of maternal PUFAs throughout the whole pregnancy. It has been previously shown that PUFA levels in plasma phospholipids do change throughout pregnancy(101). Secondly, we did not consider the influence of postnatal fatty acid exposure of the children, which also has been reported to be associated with childhood allergic diseases(102). Third, we could not rule out the possibility of misclassification as some of the exposure and outcome measurements (e.g. maternal allergy, infants' allergic diseases) were based on self or parental reported information, rather than clinical diagnosis by a medical doctor or objective measures such as IgE analyses. Subjects who did not report a positive answer at any time point but had missing data at more than two time points were classified as "missing", while those with missing data at only one or two time point were included as controls. It is acknowledged that this may lead to an overestimation of the prevalence of clinical outcomes.Moreover, the

information obtained by questionnaire did not assess in detail the severity of the outcomes and different phenotypes of clinical outcomes. Finally, as with any observational studies, we cannot rule out the possibility of residual confounding by unknown factors, even though we controlled for major known confounders.

Chapter 5. CONCLUSION

Findings from this study provide no support for the hypothesis that the risk of early childhood allergic diseases is modified by variation in maternal exposure to n-3 and n-6 PUFAs during pregnancy in an Asian population. Further follow up of the children to an older age is highly recommended. Overall, results from observation studies examining the relationship between maternal PUFAs and offspring early allergic outcomes are inconclusive. Well-conducted and sufficiently-powered dietary or supplementation trials to examine dose-response would be warranted to further investigate and validate this hypothesis.

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