THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Polyunsaturated fatty acids and allergy development

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POLYUNSATURATED FATTY ACIDS AND ALLERGY DEVELOPMENT

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Front cover: A mast cell during an allergic reaction and the fatty acid arachidonic acid (AA).

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ABSTRACT

Allergies have increased strikingly in the affluent parts of the world during the last century. The cause of the rapid increase is unknown but several risk factors have been postulated, the main ones relating to reduced microbial exposures and changed diet. In parallel with the increased prevalence of allergies, consumption of saturated fat has declined, while consumption of polyunsaturated fat has risen. The change in dietary fatty acid composition is suggested to influence the risk of developing allergy.

This thesis work aimed to investigate whether and how the fatty acid compositions in the body and in the diet affect allergy development. The fatty acid pattern in breast milk and serum was investigated in women with different allergic manifestations. Furthermore, the fatty acid pattern in cord blood was analyzed as a putative factor affecting subsequent development of allergies. In animal models, the effect of diets rich in polyunsaturated fatty acids (PUFAs) on different types of immune responses and hypersensitivity reactions was studied and the consumption of PUFAs during allergic reactions was evaluated. Since dairy farms are known to be an allergy-preventing environment, dietary differences between farmers and non-farmers were investigated.

Lactating women with an allergic phenotype that included eczema had PUFA patterns in breast milk and serum that differed from that of non-allergic women and women with respiratory allergies. Eczematous women had lower levels of several PUFAs, such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), compared to non-allergic women as well as women with isolated respiratory allergy. Fatty acid pattern in cord blood was measured in children that subsequently developed eczema or respiratory allergies. Infants who later developed allergies had higher cord blood levels of PUFAs, both from the n-3 and n-6 series, than children who stayed non-allergic. Studies in mouse models showed that a diet rich in n-3 PUFA (supplemented with fish oil) increased IgE production in an airway hypersensitivity model, but suppressed proliferation and cytokine production of T cells in a delayed-type hypersensitivity (DTH) model. During the challenge phase and the resulting allergic inflammatory reaction a significant reduction of PUFAs was monitored in both models, but most obvious in the airway model. When comparing farming and non-farming women, farmers had higher levels of saturated fatty acids (SAFAs) and lower levels of PUFAs in their breast milk. Farmers also had a higher intake of foods containing SAFAs, such as butter and whole-fat dairy products. Conversely, non-farmers had a higher intake of PUFA-rich margarine and low-fat dairy products.

In conclusion, fatty acid pattern in the body and fatty acid composition in the diet are associated with allergy development. Dietary intake of both n-3 and n-6 PUFAs appear to facilitate atopic sensitization and allergy development in both a prospective birth-cohort study and in an experimental allergy model. The PUFAs are then consumed in the body during the allergic inflammatory reaction, which may explain the low PUFA levels characterizing allergic subjects. Thus, high intake of PUFAs may predispose to allergy and a diet rich in saturated fatty acids and low in PUFAs may be one factor explaining the low allergy prevalence among children growing up on dairy farms.

Keywords: *fatty acids, polyunsaturated fatty acids, saturated fat, butter, margarine, fish oil, breast milk, serum, cord blood, farming, allergy, atopy, IgE, DTH*

Till mamma och pappa

LIST OF PUBLICATIONS

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

I. **Sara Johansson**, Agnes Wold and Ann-Sofie Sandberg. Low breast milk levels of long-chain n-3 fatty acids in allergic women, despite frequent fish intake. *Clin Exp Allergy,* 2011, 41(4), 505-515

II. Malin Barman, **Sara Johansson,** Agnes Wold, Ann-Sofie Sandberg and Anna Sandin. High levels of long-chain polyunsaturated fatty acids in cord serum predict allergy development in childhood. *Manuscript*.

III. **Sara Johansson**, Anna Lönnqvist, Sofia Östman, Ann-Sofie Sandberg and Agnes Wold. Long-chain polyunsaturated fatty acids are consumed during allergic inflammation and affect T helper type 1 (Th1)- and Th2-mediated hypersensitivity differently. *Clin Exp Immunol*, 2010, 160, 411-419.

IV. **Sara Johansson**, Malin Barman, Karin Jonsson, Agneta Sjöberg, Hilde Brekke, Agnes Wold and Ann-Sofie Sandberg. Distinct fatty acids profile in diet and breast milk of farming women. *Manuscript.*

CONTRIBUTION REPORT

Paper I: The author, Sara Johansson (SJ), participated in the study design, performed the experimental work, interpreted data and was responsible for writing the manuscript.

Paper II: SJ was involved in the study design, performed fatty acid analyses and took part in manuscript writing.

Paper III: SJ was involved in designing experiments, performed the laboratory work and interpreted data in collaboration with Anna Lönnqvist, and was responsible for writing the manuscript.

Paper IV: SJ participated in designing experiments, performed most of the laboratory work, was involved in evaluation of results and was responsible for writing the manuscript.

ABBREVIATIONS

AA	arachidonic acid
APC	antigen-presenting cell
CBA	cytometric bead array
COX	cyclooxygenase
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
DTH	delayed-type hypersensitivity
EPA	eicosapentaenoic acid
FFQ	food frequency questionnaire
HDL	high density lipoprotein
IFN-γ	interferon-γ
IL-1, 2..	interleukin-1, 2…
LA	linoleic acid
LDL	low density lipoprotein
LNA	α-linolenic acid
LOX	lipoxygenase
LP	lipoprotein
LPL	lipoprotein lipase
LT	leukotriene
MHC	major histocompatibility complex
MUFA	monounsaturated fatty acids
NEFA	non-esterfied fatty acid
OVA	ovalbumin
PCA	principal component analysis
PG	prostaglandin
PL	phospholipids
PLA_2	phospholipase A_2
PLS	partial least square
PLS-DA	partial least square with discriminant analysis
PMN	polymorphonuclear neutrophils
PPAR	peroxisome proliferator-activated receptor
PUFA	polyunsaturated fatty acids
SAFA	saturated fatty acid
SPT	skin prick test
TAG	triacylglycerol
TGF-β	transforming growth factor β
TNF	tumor necrosis factor
TX	thromboxane
VCAM	vascular cell adhesion molecule
VLDL	very-low density lipoprotein

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INTRODUCTION

Allergy denotes immunologically mediated hypersensitivity. In allergy, a harmless foreign substance elicits an immune response (sensitization), and renewed encounter of the antigen elicits inflammation that causes symptoms (hypersensitivity). Allergies may result from different types of immune reactions. IgE-mediated reactions cause atopic allergies while $CD4^+$ T cells cause delayed-type hypersensitivity (DTH) reactions termed contact allergy.

The prevalence of IgE-mediated allergies has increased enormously in the affluent parts of the world during the last century. In Sweden about 25% of the population suffers from asthma, hay fever or atopic eczema, and 15-20% have contact allergy [1]. This makes allergy one of the most widespread diseases and national economic costs. In Sweden it has been estimated at about 10 billion SEK per year [2]. Although genetic factors play a role, they cannot explain the rapid increase in allergies, i.e. a tripling of the incidence between 1970 and 1990 in Sweden and other Western countries. Several risk and protective factors have been postulated, the main ones relating to reduced microbial exposures and changed diet.

The hygiene hypothesis was put forward by Strachan in 1989 [3]. He proposed that early childhood infections were required to induce proper maturation of the immune system. Another factor proposed to underlie, or contribute to, increased allergy prevalence is changes in dietary habits. Black and Sharpe [4] suggested that a decreased intake of saturated fat and increased intake of polyunsaturated fat may be associated with the parallel increase in allergy prevalence. There is also evidence of reduced incidence of atopic eczema in children whose diet has included fish [5-7]. The farming environment has long been known to be protective against allergies [8-19]. Early and regular contact with livestock appears to be a strong protective factor [9, 12, 17], but differences in dietary habits might also play an important role.

This thesis, and the papers on which the thesis is based, investigates the role of dietary factors and fatty acid patterns in serum and breast milk in allergy development. Dietary habits that could be protective factors in farming families are studied in an epidemiological study. Associations between fatty acid profiles in serum and breast milk and different allergic manifestations, both in lactating women and children, are investigated. In addition, studies in animal models have been used to survey how diets

rich in n-3 and n-6 polyunsaturated fatty acids (PUFAs) affect different hypersensitivity reactions.

The project was conducted as collaboration between the Department of Chemical and Biological Engineering/Food Science at Chalmers University of Technology and the Department of Infectious Medicine/Clinical Bacteriology at the University of Gothenburg. The Farm Flora study is collaboration which also includes the Department of Public Health and Community Medicine/Public Health Epidemiology Unit, Institute of Medicine/Rheumatology and Inflammation Research and Department of Clinical Science/ Pediatrics at the University of Gothenburg, and Västra Götaland regional Health Care. The prospective birth cohort study was done in collaboration with the Department of Pediatrics at Umeå University and the Department of Pediatrics at Östersund Hospital.

OBJECTIVES

The overall aim of this thesis was to investigate whether fatty acid pattern in the body and fatty acid composition in the diet are associated with allergy development, with a focus on polyunsaturated fatty acids (PUFAs).

Specific aims were:

- To investigate whether and how diets rich in PUFAs (both n-3 and n-6) affect allergy development in humans and animal models.

- To survey whether PUFA pattern in umbilical cord blood relates to risk of later development of allergies.

- To investigate whether PUFAs are consumed in the body during sensitization and allergic reactions in animal models.

- To study whether the PUFA pattern in breast milk and serum could be related to allergic disease, and to evaluate whether different allergic manifestations give raise to different PUFA profiles.

- To evaluate whether there are differences in dietary habits between farming and non-farming women, to investigate whether dietary intake of fatty acids is reflected in their breast milk and to study whether possible differences in dietary habits could be related to protection against allergy development.

BACKGROUND

John Bostock [20] first described hay fever in 1819 and noted that poor people and farmers seldom suffered from this disease, which seemed to affect only wealthy people. Since then, the prevalence of allergies has increased dramatically [21]. Although the cause of allergy is unknown, a few hypotheses have been proposed. Most of them are based on factors associated with Western lifestyle.

In 1989 David Strachan postulated the hygiene hypothesis [3] to explain the inverse relationship between poverty and family size and risk of allergy development. He proposed that infections in early childhood, transmitted via contacts with elder siblings, reduced the risk of allergic disease. The hygiene hypothesis is supported by several other correlations between lifestyle and allergy. Examples of such protective factors are attendance of day-care at an early age [22, 23], exposure to pets [24-26], poverty and crowded housing conditions [27]. All of these factors are associated with high exposure to microorganisms.

Several studies have demonstrated that growing up on a farm with livestock lends powerful protection against allergy development [9-11, 13-16]. The protective mechanisms are still to be elucidated, but two hypotheses are: 1. exposure to protective microorganisms through contact with animals and through the diet, and 2. dietary factors that modulate the immune system in a protective way.

Epidemiological and experimental evidence suggests that dietary habits may influence the risk of developing allergy. Black and Sharpe [4] proposed that changed patterns of fatty acid intake in the West have contributed to the rise in allergies. Parallel to the increased prevalence of allergies, consumption of saturated fat has declined and consumption of PUFAs, essentially from the n-6 series, has risen [28, 29].

Lipids

Lipids are a broad group of naturally occurring molecules whose main biological functions include energy storage, formation of cell membranes and action as signaling molecules. Lipids are usually divided into eight categories; fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polykerides [30]. These categories can be further subdivided; in this thesis, only fatty acids, eicosanoids (both belonging to the fatty acyl group), triacylglycerols (glycerolipid group) and phospholipids (glycerophospholipids) will be further treated, with the greatest emphasis on fatty acids.

Fatty acids are the simplest lipids and are used as building blocks in triacylglycerols and phospholipids, Fig 1. Triacylglycerols are esters derived from glycerol and three fatty acids. Triacylglycerols are the major storage form of fat in the body. Phospholipids are key components in lipids bilayer of cell membranes. They consist of a diglyceride, i.e. a glycerol molecule and two fatty acids at position 1 and 2. The third OH-group, at position 3, is connected to a phosphate group which in turn is linked to cholin, serine or ethanolamine.

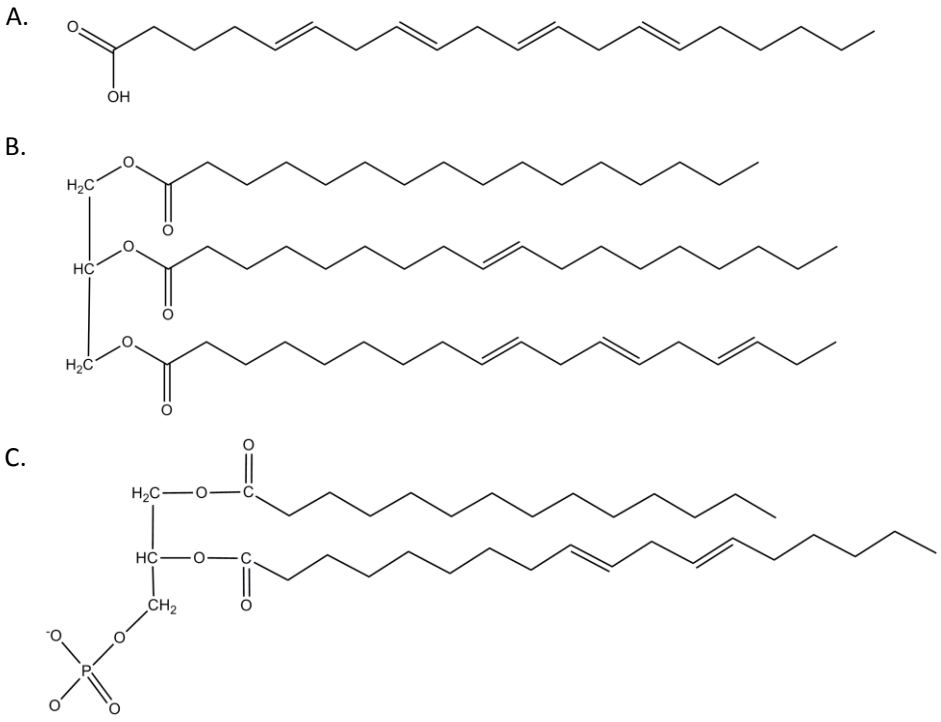


Fig. 1 A. *Free fatty acid (arachidonic acid),* ***B.*** *Triacylglycerol (1-palmitic-2-oleic-3-linolenic-triacylglycerol),* ***C.*** *Phospholipid (L-α-1-myristoyl-2-linoleoyl-phosphatidic acid)*

Fatty acids

A fatty acid consists of an unbranched hydrocarbon chain with a terminal carboxylic acid. The fatty acids are classified as saturated or unsaturated depending on whether the hydrocarbon chain contains double bonds. Saturated fatty acids (SAFAs) are straight-chain molecules with no double bonds. Fatty acids with one double bond are classified as monounsaturated fatty acids (MUFAs) and those with two or more double bonds as polyunsaturated fatty acids (PUFAs). In most of the naturally occurring unsaturated fatty acids, the orientation about double bonds is usually *cis*, rather than *trans* [31]. The orientation is important with respect to the freedom of rotation and flexibility of the

molecule. A system of abbreviation has been developed to provide a convenient way of referring to fatty acids, Table 1. The number before the colon gives the number of carbon atoms and after the colon the number of double bonds. The carbon atoms are numbered with the carboxylic terminal as number 1, and double bond locations are designated by the number of the carbon atom on the carboxylic side. The location number follows the other two numbers, e.g. n-3. Most of the naturally occurring fatty acids have an even number of carbon atoms [31].

Saturated fatty acids

In humans, fatty acids are synthesized predominantly in the liver and lactating mammary glands and, to a lesser extent, in the adipose tissue [31]. The synthesis of SAFA takes place in the cytosol and they are formed from acetyl-CoA. The major product is palmitic acid (16:0), although it is myristic acid (14:0) in mammary glands, which can be elongated to longer saturated fatty acids. These are in turn converted to MUFAs by desaturation [32]. Saturated fatty acids are also supplied by dietary intake. They are found in animal products such as meat, milk, cheese and butter.

Table 1. Nomenclature of the most common fatty acids

Systemic name	Sorthand designation	Trivial name and abbreviations
Saturated fatty acids (SAFA)		
Hexadecanoic	16:0	palmitic
Octadecanoic	18:0	stearic
Eicosanoic	20:0	arachidic
Docosanoic	22:0	behenic
Monounsaturated fatty acids (MUFA)		
cis-9-hexadecenoic	16:1 n-7	palmitoleic
cis-11octadecenoic	18:1 n-7	cis-vaccenic
cis-9-octadecenoic	18:1 n-9	oleic
cis-13-docosenoic	22:1 n-9	erucic
Polyunsaturated fatty acids (PUFA), n-6 series		
all-cis-9,12-octadecadienoic	18:2 n-6	linoleic (LA)
all-cis-6,9,12-octadecatrienoic	18:3 n-6	γ-linolenic (GLA)
all-cis-11,14-eicosadienoic	20:2 n-6	eicosadienoic
all-cis-8,11,14-eicosatrienoic	20:3 n-6	dihomo-γ-linolenic (DHGLA)
all-cis-5,8,11,14-eicosadetraenoic	20:4 n-6	arachidonic (AA)
all-cis-13,16-docosadienoic	22:2 n-6	docosadienoic
all-cis-7,10,13,16-docosadetraenoic	22:4 n-6	adrenic
all-cis-4,7,10,13,16-docosapentaenoic	22:5 n-6	osbond
Polyunsaturated fatty acids (PUFA), n-3 series		
all-cis-9,12,15-ocadecatrienoic	18:3 n-3	α-linolenic (LNA)
6,9,12,15-octadecatetraenoic	18:4 n-3	stearidonic
all-cis-8,11,14,17-eicosatetraenoic	20:4 n-3	eicosatetraenoic
all-cis-5,8,11,14,17-eicosapentaenoic	20:5 n-3	timnodonic (EPA)
all-cis-7,10,13,16,19-docosapentaenoic	22:5 n-3	clupadonic (DPA)
all-cis-4,7,10,13,16,19-docosahexaenoic	22:6 n-3	cervonic (DHA)

Uptake and synthesis of PUFAs

Most fatty acids are nonessential for humans. The exceptions are linoleic acid (LA, 18:2 n-6) and α-linolenic acid (LNA, 18:3 n-3). LA is found in for example corn, rapeseed and sunflower oils while LNA is found primarily in rapeseed, flaxseed and soybean oils. Longer PUFAs of the n-6 and n-3 families can be produced, to some extent, from LA and

LNA. The process occurs predominantly in the endoplasmic reticulum membranes. The metabolic pathways for LA and LNA involve the same enzymes, Δ6- and Δ5-desaturases, which make the two pathways connected and competing, Fig. 2.

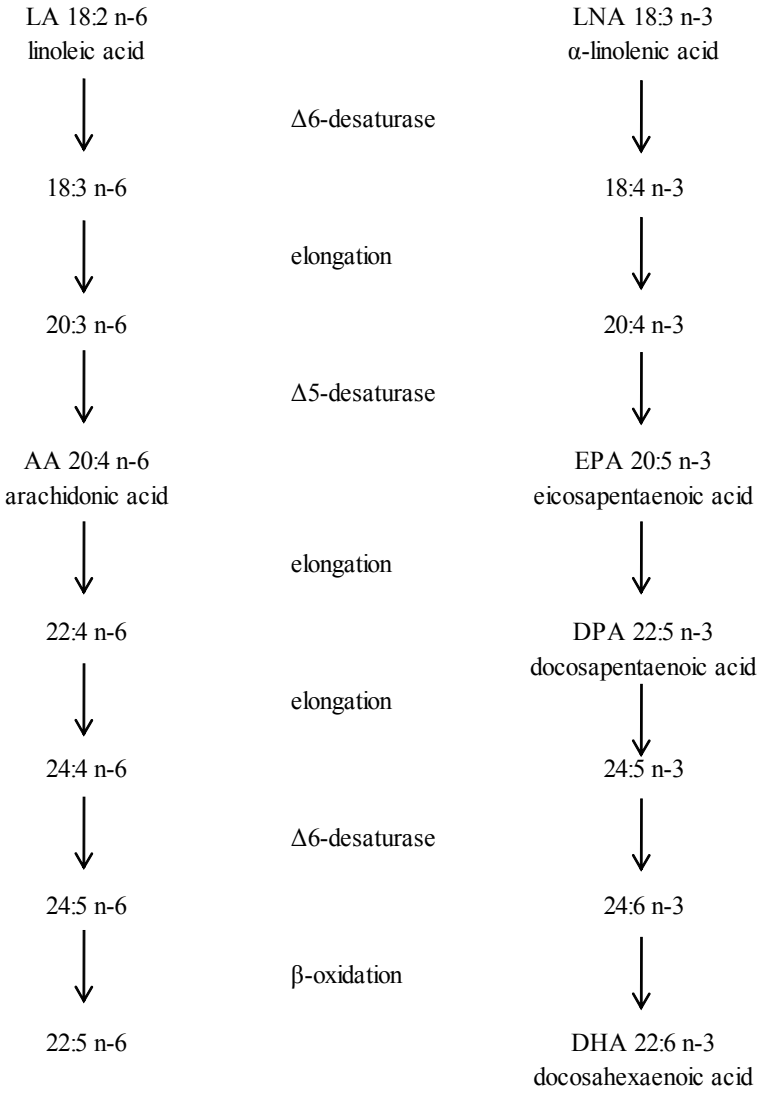


Fig. 2 *The metabolic pathway for polyunsaturated fatty acids linoleic and α-linoleic acids.*

The first step in desaturation by Δ6-desaturase activity is the rate-limiting step of this pathway. The affinity of Δ6-desaturase for LNA is greater than for LA. However, the typically high intake of LA results a in high concentration of LA and low concentration of LNA in tissue lipids and preferential conversion of n-6 PUFA [32]. Several studies have investigated the potential to convert LNA to eicosapentaenoic acid (EPA), docosapentaenoic acids (DPA) and docosahexaenoic acid (DHA). As reviewed by

Plourde et al [33] the conversion rate of LNA to EPA is ≈5%, and to DHA <0.5%. However, the capacity to synthesize EPA, DPA and DHA seem to be higher in women during pregnancy [34]. Several factors are known to affect the Δ6-desaturase activity, and therefore the conversion of LA and LNA to longer PUFAs. Examples of nutritional factors are low insulin levels and a deficiency of protein and minerals such as iron, zinc, copper and magnesium, which are often associated with malnutrition [32]. Other negatively affecting factors are alcohol consumption [35] and smoking [36].
Long-chain PUFAs can also be supplied through the diet. The n-6 fatty acid arachidonic acid (AA) is found in egg and most types of meat while n-3 PUFAs EPA, DHA and DPA are only found in marine foods, such as fish, shrimps and crayfish.

Metabolism of dietary fats

Current Swedish nutrition recommendations are that 25-30% of the total energy intake should be from fat, distributed as 10% SAFA, 10-15% MUFA and 5-10% PUFA of the total daily energy intake [37]. Fat is usually ingested as triacylglyceroles, which are hydrolyzed to glycerol, free fatty acids, as well as mono- and diacylglycerols in the lumen of the small intestine. The hydrolyze products are absorbed by the intestinal epithelial cells, where triacylglyceroles are assembled and transported to the mucosal side. Triacylglycerols and other fat molecules (cholesterol) are transported by chylomicrons, which are fat droplets, surrounded by a phospholipid layer with proteins, termed alipoproteins. The chylomicrons transport dietary fats to peripheral tissues where fatty acids are released from the triacylglycerols due to hydrolysis by lipases situated on the capillary endothelium. The fatty acids are then either combusted for energy production or recombined into triacylglycerols for storage.
Between meals, fat supplies to the tissues are provided via lipoproteins produced in the liver. These are lipid droplets, smaller in size than chylomicrons but are also surrounded by a phospholipid membrane with specific alipoproteins. Lipoprotein particles are divided according to density into very-low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL).

Placental fatty acid transport

During pregnancy fatty acids are transported from the mother´s circulation across the placenta to the fetus for use as building blocks for cellular membranes. PUFAs, especially AA and DHA, are essential for the development of many organs and cells during fetal life, including the brain and the retina [38]. The placenta lacks desaturase activity and even though isotope studies have shown fetal capacity to produce PUFAs [39], it is not known to what extent this occurs and it is generally assumed that the fetal requirements

are met by placental transfer of fatty acids [40]. The transported fatty acids can derive from free fatty acids in the maternal circulation or they are cleaved off from triacylglycerols in lipoproteins by lipoprotein lipases located at the maternal surface of the placenta.

Non-esterfied fatty acids (NEFAs) are transported from the maternal side of the placental membrane through the microvillus membrane into the syncytiotrophoblast either via diffusion [41] or carrier-mediated transport [42], Fig. 3. The most important proteins in placental fatty acid uptake are fatty acid translocase (FAT/CD36), fatty acid transport protein with acyl-CoA-synthetase activity (FATP) and plasma membrane fatty acid binding protein (P-FABPpm), Fig. 3 [43]. The lipoprotein lipases are also expressed in the cytosol of the syncytiotrophoblast, where the NEFAs can be re-esterfied and deposited as triacylglycerols for later release and transport into the fetal circulation [43].

The placenta is capable of preferential and selective transport of PUFAs to the fetus. Haggarty *et al* [44] found the order of preference to be DHA>AA>LNA>LA. It has been suggested that the selectivity depends on the ability of placental lipases to release various PUFAs from triacylglycerols [45]. The preferential and selective placenta transport might also be dependent on the selectivity of the transport proteins [43].

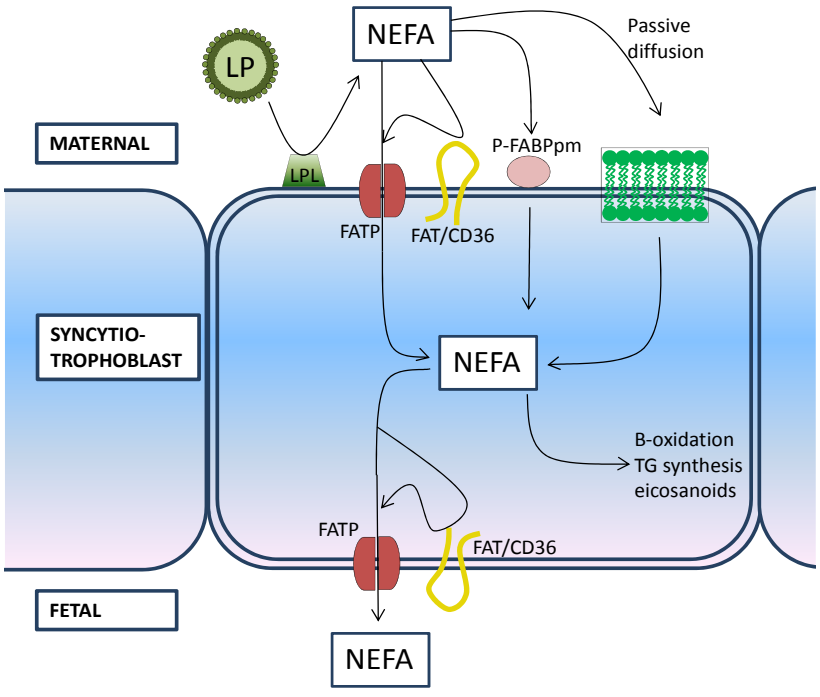


Fig. 3 *Model of placental fatty acid transport. LP= lipoprotein, LPL= lipoprotein lipase, NEFA= non-esterfied fatty acid, TG= triacylglycerols. Adapted after [43].*

Fatty acids in breast milk

After birth, breast milk is usually the infant´s first food. It is very rich in nutrients. Mature milk contains about 7% carbohydrates, mainly lactose, 0.9-1.3% protein and 3-5% fat (mainly as triacylglycerols) [46]. Colostrum contains less saturated fat and more unsaturated fat than mature milk [47] but has a lower total fat content. The fatty acids are either taken up from plasma chylomicrons through the action of mammary lipoprotein lipase, or are synthesized from glycerol and fatty acids in the alveolar cells of the mammary gland. The fatty acids derived from chylomicrons may originate either from recent dietary intake, be released from maternal adipose tissue or synthesized *de novo* in the maternal liver [48].

Biological roles of lipids

Lipids have profound effects in bodily functions. The most evident biological effects of lipids are:

- use as fuel for generation of energy in the cells
- components of cellular and intracellular membranes
- role as intracellular messengers
- substrates for production as inflammatory mediators

These functions will be briefly outlined below.

Role as nutrient

Fat is stored as triacylglycerols in adipose cells, and that is where the catabolic process starts. Triacylglycerols are hydrolyzed to yield glycerol and free fatty acids. The hydrolysis products exit the adipocytes by passive diffusion into the blood, where the fatty acids bind to albumin. They are released from albumin and taken up by cells, also by passive diffusion. Inside the cells, the fatty acids are activated by acylation of coenzyme A (CoA) to become fatty acyl-CoAs. These are transported into the mitochondria, which are the site of the β-oxidation pathway. During β-oxidation the fatty acid chains are broken apart into acetic acid fragments and yield acetyl CoA molecules which enter the tricarboxylic cycle to be oxidized to carbon dioxide, water and release energy [31].

Membrane lipids

All cellular membranes consist of phospholipids and cholesterol. The polar phosphate end and the non-polar fatty acid part of the phospholipid molecule make the phospholipids form lipid bilayers, which make them suitable for cell membranes [32]. The composition of fatty acids in phospholipids depends on our diet. There is usually a saturated fatty acid at position 1 and an unsaturated in the middle position, position 2. The most common

unsaturated fatty acid is AA, but it can also be an n-3 fatty acid such as EPA or DHA [32].

The composition of fatty acids in membranes strongly influences their fluidity. Membrane fluidity increases with an increased proportion of unsaturated fatty acids. The reason for this is that PUFA chains are extremely flexible and can rapidly change conformational states [49]. The flexibility differs significantly between n-3 and n-6 PUFAs, and the fluidity is also affected by the number of double bonds. Thus, the n-3 and n-6 PUFA composition affects the physical properties of the membranes, thereby altering protein function and trafficking.

Cellular membranes consist of micro domains called lipid rafts that are rich in saturated fatty acids, sphingolipids, cholesterol and glycosylphosphatidylinositolanchored (GPI) proteins. The rafts serve as organizing centers for the assembly of signaling molecules, influencing membrane protein trafficking and regulating receptor trafficking [50]. The composition of lipid rafts can be modified by changes in the dietary intake of PUFAs. This could affect activation of proteins within the rafts that are important in both T- and B-cell activation [50, 51].

PUFAs as intracellular signaling molecules

Peroxisome proliferator-activated receptors (PPARs) are a group of ligand-activated nuclear transcription factors belonging to the nuclear receptor superfamily. There are several isotypes of PPAR, where PPAR-γ is the type that is associated with lipids and inflammation. PPAR-γ plays an essential role in differentiation of adipocytes and in maintaining their special functions such as lipid storage, and also seems to limit inflammation by interacting with NFκβ activation. Various PUFAs, especially those of the n-3 series, are natural ligands for PPAR-γ, and changes in PUFA intake can thereby affect the intracellular signaling and the inflammatory process.

PUFAs as substrates for inflammatory mediator production

Several important inflammation mediators are formed from phospholipids, which are the major components in cell membranes. As a cell gets an inflammatory signal, the enzyme phospholipase A_2 (PLA_2) binds to cellular membranes and releases arachidonic acid which is converted via COX (cyclooxygenase) to PGH_2. This intermediate can then be converted further to prostaglandins and thromboxanes. All types of cells produce COX, but, depending on enzymes downstream of COX, they produce different types of lipid mediators when stimulated. There are two forms of COX, COX-1 is constitutively expressed, but is produced in larger amounts during inflammation. COX-2 is mostly induced during the inflammatory reaction.

Alternatively, AA is converted by 5-LOX (lipoxygenase) or 15-LOX. These enzymes are only found in inflammatory cells (e.g. granulocytes and mast cells) and are responsible for the synthesis of leukotrienes. EPA is a competitive inhibitor for production of eicosanoids from AA. Via COX-1, COX-2 and 5-LOX, EPA is metabolized to form the 3-series of prostaglandins and thromboxanes and the 5-series of leukotrienes.
Prostaglandin E_2 (PGE_2) has a number of pro-inflammatory effects including inducing fever and vasodilation and enhancing pain. It also acts anti-inflammatorily by suppressing production of TNF-α and IL-1 [52]. PGE_2 is a strong suppressor of T cell proliferation and IFN-γ production [53]. Leukotriene B_4 (LTB_4) is chemotactic for polymorphonuclear neutrophils (PMN) [54]. Thromboxane A_2 (TXA_2) promotes platelet aggregation. Prostaglandin D_2 (PGD_2) is produced in mast cells during allergic responses and asthma [52], and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) induces smooth muscle contraction [32]. Leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4) promote endothelial cell permeability and airway smooth muscle constriction during anaphylactic reactions and asthma.
A novel group of EPA- and DHA-derived metabolites has recently been identified. The EPA-derived mediators are termed E-series resolvins and the DHA-derived D-series of resolvins and protectins (neuroprotectins D1). The novel mediators are suggested to be anti-inflammatory since they are shown to regulate trafficking and migration of inflammatory cells and to block transendophelial migration of leukocytes. They are also suggested to regulate the levels of pro-inflammatory peptide mediators by inhibiting dendritic cell migration and cytokine release. [55, 56].

Diet and allergy development

Several dietary components have been investigated in relation to allergy development, but most evidence has been gathered for three dietary components:

- butter and margarine
- fish
- supplements with fish oil or isolated n-3 PUFAs

Butter and margarine

In 1997 Black and Sharpe [4] suggested that the increased intake of n-6 PUFAs as well as decreased intake of n-3 PUFAs and SAFAs may have contributed to the increased prevalence of allergic disease in Western countries. The theory agrees with subsequent reports concerning the unification of former East and West Germany, where the prevalence of allergies increased rapidly in the eastern part of Germany. This increase occurred coincidentally with a change towards a Western lifestyle, including increased

intake of margarines rich in n-6 PUFAs such as LA [57]. An increase in margarine intake usually means a decrease in butter intake, and in 2001 a Finnish study reported that children with atopic eczema or rhinitis ate more margarine and less butter than non-allergic controls [58]. The positive association between margarine intake and allergy has subsequently been shown in several studies [59-62], and the suggested explanation is effects of the high PUFA content.

Fish

A protective effect from maternal fish intake during pregnancy has been shown on eczema [62, 63] and asthma [64] in their offspring. Early introduction of fish into the child´s diet has also repeatedly been shown to be associated with less development of eczema [5-7] and other atopic diseases [5]. Another inverse association between atopy and fish intake is that asthmatic subjects eat less fish than non-allergic ones [63, 65], although, some studies have shown negative effects of fish on allergy [66, 67], and a few studies observed no associations [68-70].
Fish and other marine foods are major sources of the n-3 PUFAs EPA, DHA and DPA, and increased consumption of seafood leads to an increased proportion of n-3 PUFAs in cell membranes. Other seafood-derived bioactive components that could play a role in modulating the risk of allergy development include e.g. vitamin D [71] vitamin E [68] and selenium [72].

Supplements with fish oil or isolated n-3 PUFAs

Numerous studies have investigated effects of fish oil or pure n-3 fatty acid supplementation on allergy development and reduction of allergic symptoms. After fish oil supplementation during pregnancy, Furuhjelm *et al* [73] reported a lower incidence of atopic eczema and food allergy in the infants compared to the placebo group who received soy oil rich in n-6 LA. Others show no difference in atopy development in the infants [74-76] but less severe eczema symptoms after fish oil supplementation [74, 75]. Koch *et al* reported decreased SCORAD (SCORing Atopic Dermatitis), i.e. less severe eczema symptoms, in eczematous adults after supplementation with isolated ethyl esters of DHA [77].

Olsen *et al* reported a reduction of asthma in children whose mothers received fish oil supplement during pregnancy compared to children whose mothers were given olive oil [78]. Interestingly, they had a second control group that received no supplements. The children in the second control group exhibited diagnostic patterns remarkably similar to the group whose mothers had been given fish oil supplements, i.e. children in the olive oil

group had a higher prevalence of asthma than children in the fish oil and no supplement groups. These results indicate a possibility that olive oil supplementation could, in fact, have increased the risk of allergy development in the child.

Mishrshahi *et al* [79, 80] and Almqvist *et al* [81] studied children at high risk of developing atopic disease, i.e. had a family history of allergy. After supplementation with fish oil and use of canola-based oils and spreads (high in n-3 PUFAs, low in n-6 PUFAs) in all food preparation from six months of age, they found no difference in atopy prevalence at 18 months and five years, respectively. The former group, Mishrshahi *et al*, did follow-up studies on the high-risk children at three [82], five [83] and eight [84] years, with no observed effects of fish oil on any clinical outcome relating to allergy.

In all studies mentioned above, the control group was given placebo supplementation of oils rich in PUFAs; soy oil, sunflower oil or olive oil, except in Koch´s study where the control group got saturated fatty acids; a mix of caprylic acid (8:0) and capric acid (10:0). Soy oil and sunflower oil contain high levels of PUFAs, mainly n-6 LA, and olive oil contains primarily MUFAs such as 18:1 n-9, but also about 10% PUFAs of which most are n-6 PUFAs. In future studies it should be considered how to use n-6 PUFA-rich oils as placebo supplementation in control groups, since the effects on allergy is not completely defined.

PUFA pattern and allergy development

Breast milk PUFA pattern in relation to allergy development

Breast milk is the major nutritional source for most infants, and allergies often develop in early infancy or childhood. Therefore relationships between PUFA composition of breast milk and development of allergy have been studied repeatedly. The studies are compiled in Table 2. With regard to colostrum, some show no fatty acid differences, but two studies report higher DPA and one report higher DHA in breast milk received by children who subsequent developed atopic eczema. One study also reported higher LA in breast milk given to children who later developed atopic rhinitis.

When studying fatty acids in mature breast milk which were suckled on of infants who later became allergic, the results are also inconsistent. Some report lower levels of EPA, other report higher or lower levels of DPA in breast milk received by subsequent allergic infants. Further, one study showed higher content of LNA and another study showed lower LA in breast milk given to future allergic children.

Hence, the results reported on associations between PUFAs in breast milk and subsequent development of allergy in children is highly inconsistent.

Table 2. Fatty acid differences in breast milk received by children who later developed atopy

Reference	Subjects	Atopic criteria	Controls	Sample	Fatty acid differences
[85]	22 children with atopy	Clin.hist., SPT	30	3 months	Atopics received higher LNA and 20:3 n-6
[86]	24 children with atopy	SPT	15	Colostrum	No FA differences
[86]	21 children with atopy	SPT	61	1 and 3 months	Atopics received lower EPA at 1 month, and lower DPA at 3 months
[87]	20 children with atopy	Clin.hist., SPT	20	3 months	No FA differences
[88]	17 children with atopy	IgE, RAST	60	Colostrum	No FA differences
[89]	29 children with atopic eczema	SPT	148	Colostrum	Atopics received higher DHA and DPA
[90]	13 children with atopy	SPT, Hanifin	21	1 month	Atopics received lower EPA
[91]	11 children with atopic eczema	Clin.hist., SPT	27	3 months	Atopics received lower LA
[92]	6 children with atopic ezema	Clin.hist., SPT, Hanifin	19	6 months	No FA differences
[93]	27/ *15* children with atopic eczema	Clin.hist., SPT	172/ *105*	Colostrum/ *3 months*	Atopic received higher DPA
[93]	37/ *25* children with atopic rhinitis	Clin.hist., SPT	168/ *100*	Colostrum/ *3 months*	Atopics received higher LA in colostrum and higher DPA at 3 months

Clin.hist.= clinical history, SPT= skin prick test, RAST= radioallergosorbent test, Hanifin= eczema diagnosed by Hanifin and Rajka criteria, FA= fatty acid

Breast milk PUFA pattern in relation to maternal atopy

The PUFA composition of breast milk will be influenced by a number of factors including maternal diet and maternal metabolism, both which may be affected by atopic disease. The PUFA pattern in breast milk from atopic and non-atopic women has been investigated in several studies, to evaluate if children nursed by atopic and non-atopic women receive different fat qualities. The reports are summarized in Table 3. The results are rather inconsistent, but a few studies report no fatty acid differences and some report lower n-3 PUFAs in breast milk from atopic mothers. However, another study shows higher DHA in breast milk from atopic mothers.

Table 3. Fatty acid differences in breast milk from atopic and non-atopic mothers

Reference	Subjects	Atopic criteria	Controls	Sample	Fatty acid differences
[94]	17 atopic mothers	Clin.hist.	17	1 month	Atopics had lower EPA, DHA, DPA and 20:3 n-6
[87]	20 atopic mothers	Clin.hist.	20	3 months	Atopics had higher DHA, but lower 18:3 n-6
[95]	168 atopic mothers	Clin.hist., IgE	107	3 months	No FA differences
[96]	43 allergic mothers	Clin.hist.	51	2-3 months	No FA differences
[89]	107/ *60* atopic mothers	Clin.hist., SPT	55/ *36*	Colostrum/ *3 months*	No FA differences
[97]	144 atopic mothers	Clin.hist.	14	1 month	Atopics had lower EPA

Clin.hist.= clinical history, SPT= skin prick test, FA= fatty acid

Serum PUFA pattern in relation to allergy

Allergies may manifest within months after birth. This indicates that the immune dysregulation occurs very early during development. A number of studies have therefore investigated the PUFA pattern in serum from umbilical cord blood as a possible predictor of subsequent allergy. In table 4 studies evaluating PUFA pattern in cord blood are summarized. Part A includes studies on cord blood from infants who later developed allergies, and in two of these no differences in PUFA composition was observed, while in the third lower AA and DHA but higher LA was reported in subsequent allergic children. In part B studies including infant with a family history of allergies are compiled. According to the reports concerning infants of allergic mothers, they have higher levels of AA, EPA and DHA in cord blood. This may indicate that something related to the allergic disease has an impact on PUFA supply to the fetus.

Several studies have also investigated PUFA patterns in serum from children who have developed atopy compared to non-atopic children. The rather inconsistent results are summarized in table 5. However, some trends in the different results can be seen. The fatty acid 18:3 n-6 is reported to be low in serum phospholipids in atopic children in a couple of studies, and according to some reports DHA is lower in serum cholesteryl esters in children with atopic disease.

Table 4. Fatty acid differences in cord blood from children who A. developed atopy later on, B. had a family history of atopy

Reference	Subjects	Atopic criteria	Controls	Sample	Fatty acid differences
A.					
[98]	9 infants who later developed atopic dermatitis	IgE	107	Serum PC	Higher LA, but lower AA, DHA and 20:3 n-6 in subsequent atopic children
[86]	19 infants who later developed atopy	Clin.hist., SPT	40	Serum PL	No FA differences
[99]	35 infants who were atopic at 3 y	SPT	35	Whole plasma	No FA differences
B.					
[100]	33 infant with allergic mothers	Clin.hist., IgE	35	Serum PL	Higher AA, EPA, DHA and 20:3 n-6 in high-risk infants
[101]	25 infants from allergic mothers	Clin.hist., IgE	22	Serum PL	Higher AA, EPA, DHA and 22:4 n-6 in high-risk infants
[102]	50 infants with family history of atopy	Clin.hist.	50	Plasma PL	Lower AA and 22:4 n-6 in high-risk infants

Clin.hist.= clinical history, SPT= skin prick test, FA= fatty acid, PL= phospholipid, PC= phosphatidylcholine

Table 5. Fatty acid differences in serum from atopic and non-atopic children

Reference	Subjects	Atopic criteria	Controls	Sample	Fatty acid differences
[86]	16 atopic infants	Clin.hist., SPT	35	Serum PL	Atopics had higher levels of 22:4 n-6 and 22:5 n-6
[87]	20 atopic infants	Clin.hist., SPT	20	Serum PL, TG and CE	Atopics had lower DHA in CE and 18:3 n-6 in PL. Atopics had higher LA in TG.
[58]	126 children with atopic eczema	Doctor´s diagnosis	126	Serum CE	Atopics had lower EPA and DHA
[58]	145 children with atopic rhinits	Doctor´s diagnosis	145	Serum CE	No FA differences
[58]	47 children with asthma	Doctor´s diagnosis	47	Serum CE	No FA differences
[103]	8 children with atopic rhinitis and/or asthma	Clin.hist., IgE	6	Plasma	Atopics had lower 18:3 n-6
[92]	6 children with atopic eczema	Clin.hist., SPT, Hanifin	19	Serum PL	Atopics had lower 18:3 n-6, but higher EPA

Clin.hist.= clinical history, SPT= skin prick test, Hanifin= eczema diagnosed by Hanifin and Rajka criteria, FA= fatty acid, PL= phospholipid, TG= triacylglycerol, CE= cholesteryl ester

Potential phases of action of PUFAs on immune functions

Allergy denotes immune mediated hypersensitivity, defined as the appearance of symptoms when a previously sensitized individual is exposed to the antigen to which she/he has developed an immune response. The proposed effects of PUFA intake on allergy could occur on one or several of the following levels:

- the sensitization phase
- expansion and maturation of T lymphocytes
- expansion of B cells and maturation into IgE-producing plasma cells
- the symptom phase in which blood vessel tonus and permeability are affected and inflammatory cells are recruited into the tissues

The phases will be briefly described below as a background for understanding the potential immune modulating effects of PUFAs.

Sensitization phase

Adaptive immune responses start with presentation of the antigen by an antigen-presenting cell (APC), usually a dendritic cell, to naïve T cells. This presentation occurs in the lymph nodes to which the antigen is brought via the lymph, or carried within dendritic cells from mucosal membranes or the skin. Naïve T and B cells circulate through the lymph nodes in search of their specific antigen.

Naïve $CD4^+$ T cells (termed helper cells) recognize and interact with peptides presented on MHC II molecules, become activated, and start to proliferate. They can then differentiate into various effector subsets, Fig. 4. The route of differentiation is thought to depend on the antigen, the activation state of the APC, and the surrounding environment.

Mature and activated T cells are termed effector cells and participate in immune responses aiming to eliminate the offending antigen and resolve the infection. After this initial immune response, most of the effector cells die, while a fraction survive and become long-lived memory cells.

The Th1 differentiation pathway is stimulated by bacteria and strong adjuvants such as Freund´s complete adjuvant (mineral oil containing dead mycobacteria). Activated APCs secrete IL-12 which stimulates maturation of Th1 cells. Th1 cells produce the cytokine IFN-γ which further induces IL-12 production from APCs. IFN-γ also activates the killing capacity of macrophages. Th1 cells are responsible for delayed-type hypersensitivity, which is a hallmark of contact allergy. A person who has memory Th1 cells against a contact allergen is termed sensitized and can develop symptoms upon renewed encounter with the same antigen.

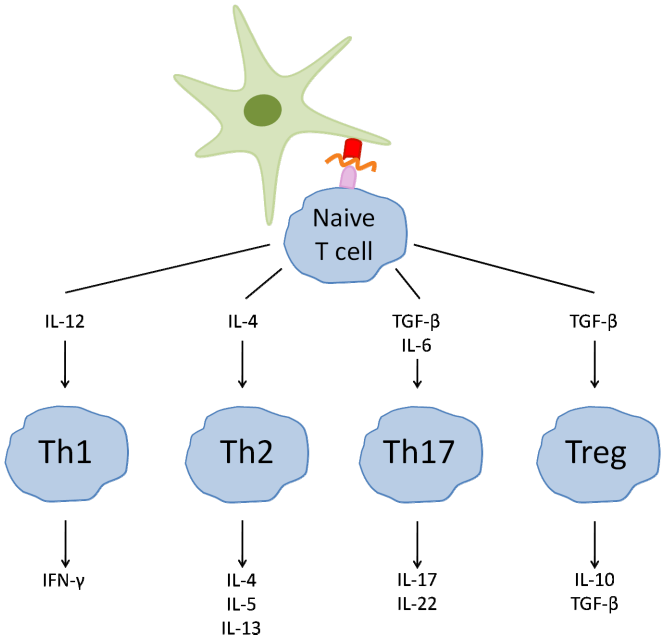


Fig. 4 *T cell subsets. A naïve $CD4^+$ T cell can differentiate into diverse effector cells (Th1, Th2, Th17 and T reg) upon activation, which are influenced by signals, such as cytokines from the antigen-presenting cell and the environment.*

The Th2 pathway is activated in response to helminthes and allergens and by the adjuvant alum ($Al(OH)_3$). Th2 cells produce IL-4, which is a growth factor for Th2 cells. IL-4 also activates B cells to produce antibodies. Other cytokines produced by Th2 cells are IL-13, which enhance IgE production, and IL-5, which increases the production of eosinophils in the bone marrow.

Other T cell subsets include Th17 cells that are involved in neutrophil recruitment and the production of defensins, antimicrobial peptides, by skin and mucosal epithelium. Regulatory T cells (Treg) inhibit immune responses by blocking activation and functions of other effector T cells [104].

The signals from the APCs that are responsible for the preferential maturation of the naïve T cells along the various pathways exemplified above are poorly known. Production of IL-12 is inhibited by bacterial products [105] and favors maturation along the Th1 pathway. Production of PGE_2 has been linked to maturation of Th2 cells and vitamin A metabolites, and the cytokine TGF-β for maturation of regulatory T cells.

IgE production

IgE is produced by plasma cells located in lymph nodes. Plasma cells derive from antigen-stimulated B cells that have been stimulated by recognition of their specific antigen and have received help from activated helper T cells. Naïve B cells produce only

IgM, but renewed or continuous antigen stimulation leads to a change in the immunoglobulin isotype to IgG, IgA or IgE. This is termed “switch” and depends on signals supplied by activated helper T cells, in the case of Th2 cells, IgE. A person who produces IgE antibodies against an allergen is termed sensitized. Sensitization is a prerequisite for the appearance of symptoms upon renewed encounter with the allergen.

The symptom phase of allergy

Allergy is immunologically implied hypersensitivity towards a harmless antigen (allergen). Allergies may originate from different types of immune reactions, either immediate hypersensitivity or delayed-type hypersensitivity (DTH).

A. Immediate/IgE-mediated allergy

The immediate type of allergy is also called atopic allergy and is mediated by IgE antibodies. IgE antibodies bind to mast cells via their membrane bound Fcε-receptor. When allergens are inhaled or ingested, it binds to the IgE antibodies, and mast cells are activated and start to degranulate. Histamine is secreted and gives rise to vasodilation and increased vascular permeability. Activated mast cells also start producing leukotrienes C_4, D_4 and E_4 from AA, which contract smooth muscles in the bronchi and initiate mucus release from mucus-producing cells. Mast cells, T cells and macrophages in the inflammatory focus produce cytokines which recruit eosinophils and T cells to the inflammatory site. Eosinophils degranulate and also synthesize a range of different lipid mediators. However, very little is known about the functions of the eosinophilic granula substances or the lipid mediators. Examples of IgE-mediated allergic manifestations are asthma, hay fever and some food allergies.

B. Contact allergy (DTH)

Contact allergy is an example of delayed-type hypersensitivity, DTH, in which Th1 lymphocytes and the Th1 cytokine IFN-γ are important actors. Small molecules that can cross the skin barrier, and to which the individual has developed memory Th1 cells, elicit activation of these T cells. Th1 cells produce IFN-γ that in turn activates tissue macrophages with increased production of chemotactic cytokines and accumulation of more T cells and monocytes in the tissue.

Potential immunomodulating effects of PUFAs

Membrane fluidity and lipid rafts

When the body experiences an alteration in fatty acid supply, the composition of the membrane phospholipids of immune cells is modified. If the membrane proportion of n-3 PUFAs increases, the n-6 PUFAs decrease. An altered PUFA composition in phospholipids changes the fluidity of the membranes. This affects receptor functioning, the activity of membrane bound enzymes and signal-transduction mechanisms [106].
It is proposed that changes in so called lipid rafts are significant for activation of Th1 cells, in contrast to Th2 activation. Lipid rafts are micro domains in plasma membranes that are platforms for cell activation and signaling between cells [50]; due to changes in PUFA composition in lipid rafts, it affects T cell responses [51].

PPAR-γ activation

The PPAR-γ transcription factor is activated by n-3 PUFAs. PPAR-γ acts in an anti-inflammatory way by interacting and disturbing the NFκβ activation. It also stimulates the degradation of inflammatory eicosanoids and inhibits production of TNF-α, IL-1β, IL-6, IL-8, VCAM-1, acute phase proteins and COX-2 [107, 108].

Affects on antigen-presenting cells

Dendritic cells (APCs) treated with DHA have been reported to have a reduced ability to activate naïve $CD4^+$ T cells [109]. The expression of transcription factors involved in differentiating pathways of Th1 (Tbet) and Th17 (RORγt) cells was reduced by DHA treatment, and production of IFN-γ and IL-17 were also reduced. However, the expression of the transcription factor involved in differentiation into Treg cells (FoxP3) was increased.
APCs which mature in the presence of AA-derived PGE_2, produce high levels of IL-10, but no IL-12 [110, 111]. The PGE_2-APCs promoted the development of Th cells that produced high levels of IL-4 and IL-5. The results suggested that elevated levels of PGE_2 promote the Th2-mediated immune response.

Effects on inflammatory responses

Meijerink *et al* [112] investigated the immunomodulating effects of the DHA metabolite docosahexaenoylethanolamide (DHEA). They showed that DHEA reduced Th1-mediated inflammation through decreased production of the chemokine MCP-1 and the vasodilating compound NO.

Intake of n-3 PUFAs has also been shown to interfere with thrombocyte activation and coagulation, which are intimately linked to inflammation and immunity, especially in Th1-driven immune reactions [113].

Concluding remarks

Altogether, dietary intake of PUFAs and PUFA pattern in serum and breast milk seem to be associated with allergy or allergy development. The most consistent result regarding dietary intake of PUFAs is the high intake of margarine and low intake of butter among allergic subjects. Several studies report associations between high fish intake and low prevalence of allergies, however reports with contradictory results are also proposed. Supplementation with fish oil or isolated n-3 PUFAs also show diverse results with respect to allergy development.

Concerning studies investigating PUFA pattern in breast milk and serum in relations to allergy development or maternal atopy the results are also fairly inconsistent. Many surveys include subjects with different allergic manifestations, the groups of participants often include low numbers of subjects and sample collection occurs at different times or ages. These factors might affect the PUFA pattern, differences between the groups and makes it hard to compare different studies with each other.

The overall inconsistency of reported associations between PUFAs and allergies opens up to further investigation of these presumed relations.

STUDY DESIGN AND METHODOLOGICAL CONSIDERATIONS

Cohort I (Paper 1)	Cohort II (Paper 2)	Cohort III (Paper 4)
Questions		
1 Do allergic and non-allergic women have different PUFA patterns in breast milk and are these patterns related to the diet?	**1** Do allergic manifestations give rise to different PUFA profiles in serum?	**1** Are there differences in dietary habits between farming women and non-farming women, and are such differences related to protection against allergy development?
2 Do different allergic manifestations give rise to different PUFA profiles in breast milk and serum?	**2** Can PUFA pattern in umbilical cord blood predict the risk of subsequent allergies?	**2** Are there differences in breast milk and serum PUFA pattern from farming and non-farming mothers and children?
Subjects		
Lactating women with different allergic manifestations	Children with different allergic manifestations	Farming/Non-farming mother-infant pairs
Methods		
Fatty acid analyses		
1 month: Breast milk Serum	**Birth:** Umbilical cord blood **13 years:** Serum	**Birth:** Umbilical cord blood **1 month:** Breast milk Maternal serum **4 months:** Breast milk Child serum
Dietary analyses		
1 month: Food frequency questionnaire[1]	**13 years:** Food frequency questionnaire[1]	**Pregnancy:** Food frequency questionnaire[1] **1 month:** 24-h dietary recall[1,2] 24-h dietary record[1,2] **4 month:** 24-h dietary recall[1,2] 24-h dietary record[1,2]

[1] Food components

[2] Nutrients

Animal models (Paper 3)	
Questions	
1. Do diets supplemented with n-3 or n-6 PUFAs affect different allergic immune reactions and how? **2.** Are PUFAs consumed in the body during allergic reactions?	
Animals	
Mice fed diets supplemented with fish oil (rich in n-3 PUFAs) or sunflower oil (rich in n-6 PUFAs)	
Models	
DTH model (Th1)	***Airway hypersensitivity model (Th2)***
Footpad swelling	Eosinophil infiltration in lungs
In vitro proliferation	Total serum IgE
Cytokine production	Specific IgE in serum
Serum fatty acids continously	Serum fatty acids continously

The thesis is based on four separate studies that include three human studies and one animal study. Dietary habits in farming families and the relation to protection against allergy are investigated in a birth-cohort study. In the retrospective case-control study PUFA pattern in umbilical cord blood is studied, and in the remaining human study fatty acid patterns in breast milk and serum are studied in relation to different allergic manifestations. Associations between these breast milk and serum fatty acid pattern and dietary intake of fatty acids are also evaluated. In the animal study, effects on different immune reactions in diets rich in n-3 and n-6 PUFAs are compared.

Study design

Cohort I (Paper 1)

This study was designed to investigate whether PUFA pattern in breast milk and serum differ between allergic and non-allergic women, and to evaluate whether different allergic manifestations give raise to different PUFA profiles. The study population comprised 45 women who gave birth between September 2005 and September 2007, 18 of whom were from the city of Göteborg in southwestern Sweden and 27 living in a rural area around 100 km from Göteborg. The latter 27 women were originally recruited to the FARM FLORA study (described under Cohort III). The women were enrolled at the respective antenatal clinics and filled in a questionnaire that attempted to identify doctors diagnosed allergic disease. Women with a clear history of asthma, allergic rhinitis and/or atopic eczema were included, as were a number of non-allergic women without allergic symptoms. To confirm the atopic/non-atopic state, a blood sample was drawn and analyzed for total IgE and specific IgE antibodies.
Blood and breast milk samples were taken one month postpartum for fatty acid analyses, and all women completed a food frequency questionnaire (FFQ) on dietary habits during pregnancy and lactation.

Cohort II (Paper 2)

This study was designed to assess allergy development in a prospective birth cohort of about 1200 children, all living in Jämtland in the Northern part of Sweden. They were born during the period February 1996-January 1997 (described in detail in [114]).
At the age of 13, three groups of subjects were selected to participate in the retrospective case-control study. The subjects were selected on the basis of allergic disease diagnosis, since the objectives were to study fatty acid pattern in serum in relation to different allergic manifestations. 44 children with atopic eczema were included; 58 children with respiratory allergy (hay fever and/or asthma) and 52 non-allergic controls with negative skin prick test and no history of atopic diseases. Blood samples had been taken at birth

(umbilical cord blood) and stored at the Department of Pediatrics at Östersund Hospital. For this study, a blood sample was collected at 13 years of age for fatty acid analyses.

Cohort III (Paper 4)

The FARM FLORA study is a birth-cohort study that enrolled 66 children born September 2005-August 2008 where the objective was to investigate why the farming environment protects against allergy development. Two potential explanations were studied in detail:

- The farming environment is characterized by increased exposure to microorganisms, through contact with animals and through food. This would accelerate the maturation of the infant´s immune system and thereby facilitate the development of tolerance against food and other harmless substances.
- Foods that are consumed on small family farms reduce inflammation and/or modulate the immune system in a way that protects against allergy development, e.g. by their fatty acid composition or other bioactive compounds in foods.

The families lived in a rural area around 100 km from Göteborg. Mothers-to-be were recruited at antenatal clinics and via advertisement in regional daily newspapers and farming trade magazines. Upon recruitment, they filled in questionnaires concerning the farm, living conditions, siblings, pets, smoking habits, allergies in the family and food frequencies during pregnancy. Blood samples were taken from the parents to analyze IgE antibodies. Blood, saliva and feces samples were collected from the children according to Fig. 5. Dietary anamneses were taken from mothers during lactation, and from children, as soon as they started to eat solid foods. During dietary assessment of the children, samples of all ingested foods during 24 h were collected.

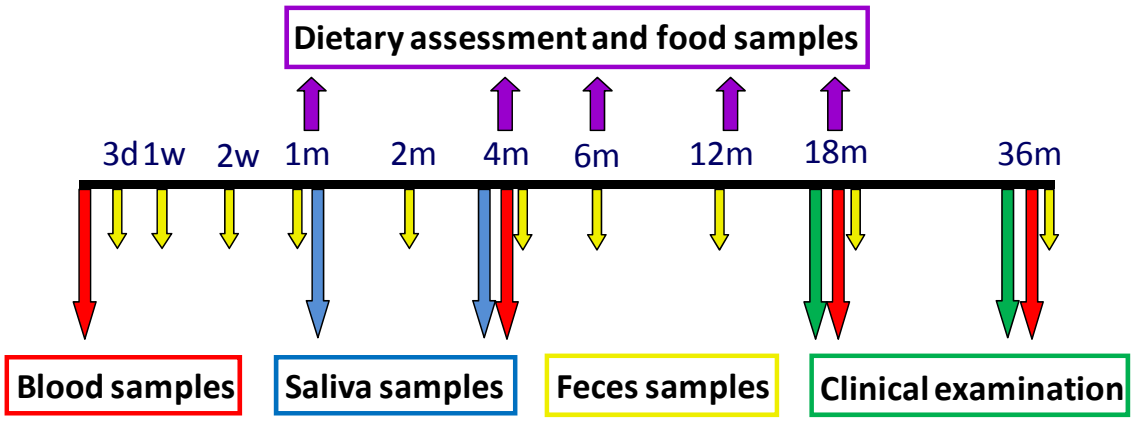


Fig. 5 *Study design in the Farm flora study*

In the work reported in paper 4, the following samples were analyzed concerning fatty acid status, composition and intake: (i) blood samples from birth (umbilical cord) and four months, (ii) breast milk from one and four months, (iii) dietary data from pregnancy (FFQ), one and four months.

Animal models (Paper 3)

Mice were fed diets supplemented with fish oil (rich in n-3 PUFAs) or sunflower oil (rich in n-6 PUFAs) or a control diet of regular mouse chow. The effects of the different diets were evaluated in two allergy models. Th1-mediated allergic reactions were studied in a delayed-type hypersensitivity (DTH) model, while Th2 reactions were surveyed in an eosinophil-mediated airway hypersensitivity model. Dietary intake was monitored by fatty acid analyses of continuous blood sampling.

In the DTH model, a Th1-mediated reaction takes place, in which the main actors are Th1 lymphocytes and the cytokine IFN-γ. NK cells, IL-12 and TNF also play key roles in this type of hypersensitivity reaction. Sensitization in the DTH model was measured by excising draining lymph nodes and stimulating the lymphocyte cells with the model antigen (OVA). Cell proliferation was measured after 7 days, and cytokine production was analyzed in the supernatant from the cell suspensions.

The airway model simulates an immediate type of IgE-mediated allergy, which involves mast cells and eosinophils, among others. In this model, sensitization was measured as OVA-specific IgE and total serum IgE. Infiltration of eosinophils into the lungs was also measured.

Fatty acid analysis

The PUFA pattern was analyzed in breast milk and serum samples using a direct transesterfication method, where both esterfied and free fatty acids were directly converted into methyl esters without prior fat extraction [115]. The direct-methylation technique had several advantages over traditional methods including fat extraction. It was less time-consuming, and gave a more complete recovery since fatty acids were directly freed from the different lipid classes of the samples. Because of the single-step procedure, there were fewer steps in which lipids could be lost and there was no need to add antioxidants since auto-oxidation does not occur after methylation [115].

In human serum, PUFA composition was analyzed in the phospholipid fraction since it reflects the long-term fatty acid intake while measurement in total lipids in serum gives a picture of the most recent intake. Phospholipid composition was determined by extracting fat from serum with chloroform and methanol [116] and then obtaining the phospholipids by separation on aminopropyl solid phase extraction columns [117].

Whole mouse blood was analyzed regarding PUFA composition in the animal model, foremost because of the small sample volume.
Breast milk PUFAs were analyzed in the total lipid fraction by the same direct transesterfication method described above.

Dietary assessment

Dietary data were collected using three different methods. Dietary habits during pregnancy (Paper 1, 4) and at 13 years of age (Paper 2) were evaluated by food frequency questionnaires (FFQ) based on the Northern Swedish 84-item FFQ [118]. Maternal food and nutrient intake during lactation (Paper 4) was surveyed using a combination of 24-h dietary recall and 24-h dietary record. All three methods have advantages (+) and limitations (-).

FFQ:
+ gives information covering a long time interval
+ registers dietary habits
+ is insensitive to intra-individual, day-to-day variation in food and nutrient intake
- risks over- and underestimation
- relies on memory
- relies on perception of serving sizes

24-h dietary recall:
+ difficult to bias because of unannounced interviews
+ guidance by the interviewer to minimize memory biases
- limited ability to register habits
- relies on perception of serving sizes

24-h dietary record:
+ easy to remember because of registration concurrent to intake
+ possibility to use kitchen scales or measuring tools to estimate weights or amount in an accurate way
+ easy to report exact estimations with respect to brands, content and preparation
- risk of adapting the diet to the better at the registration day

By using combinations of all three dietary assessment methods, our aim was to maximize the chance of recording differences between farmer´ and non-farmer´ diets and of

obtaining high quality data (i.e. giving more emphasis to dietary patterns recorded by more than one method).

Quantification of immune mediators

Specific and total IgE

OVA-specific IgE were analyzed in serum from mice included in the airway hypersensitivity model to measure sensitization. Titres of OVA-specfic IgE were assayed by passive cutaneous anaphylaxis [119] using Sprague-Dawley rats (Scanbur AB).
Total serum IgE concentrations were determined by sandwich ELISA (BD Biosciences Pharmingen).

In vitro proliferation assay

To measure sensitization in the DTH model, lymph nodes were excised and single cells were suspended and stimulated with OVA. After two days of incubation, supernatant was collected for cytokine analyses and cell proliferation was measured after seven days.

Cytokines

Cytokines (IFN-γ, TNF and IL-6) were assayed in supernatants from cell suspensions of excised lymph nodes in the DTH model using cytometric bead array (CBA; BD Biosciences, San Jose, CA, USA). CBA is a method in which several different cytokines are measured at the same time in the same sample. It is less time-consuming and more suitable for small sample volumes than are conventional methods such as ELISA.

Statistical analysis

Multivariate analysis

Multivariate analyses are particularly suitable for analyzing large data sets consisting of numerous variables. With principal component analysis (PCA) an initial over-view of possible clustering of observations is obtained on the basis of all variables simultaneously. The original set of correlated variables is combined into a small number of new variables, termed “latent variables”, “principal components” or “score vectors”, each of which accounts for as much of the variance of the original data as possible and which are also not correlated to one another [120]. In projections to latent structures by means of partial least square (PLS), the “latent variables” that co-vary maximally with a selected Y variable, for example a certain clinical condition, are searched for. PLS is then a regression extension of PCA [121]. By using PLS with discriminant analysis (PLS-DA), predetermined classes of observations are separated on the basis of all variables. The

variables that contribute to this separation can then be identified. These methods permit an evaluation of differences in variables without the risk of problems with mass significance, the need for normal distribution of data and the need for strict independence among variables, which are caveats of conventional statistics when used with this type of data.
Multivariate statistical analyses were performed using Simca-P+ 12.0 (Umetrics, Umeå, Sweden).

Univariate analysis

Univariate analyses were performed with SPSS 15.0 (Papers 1 and 3) and with PASW Statistics 18.0 (Papers 2 and 4). The Mann Whitney U-test was used to determine group differences concerning dietary data, fatty acids in serum/breast milk and immune mediators, etc., that were not normally distributed. Spearman´s rank correlation was used to test for associations and Wilcoxon´s signed-rank test for within-individual differences in serum FA measured at different time points. P values < 0.05 were considered significant.

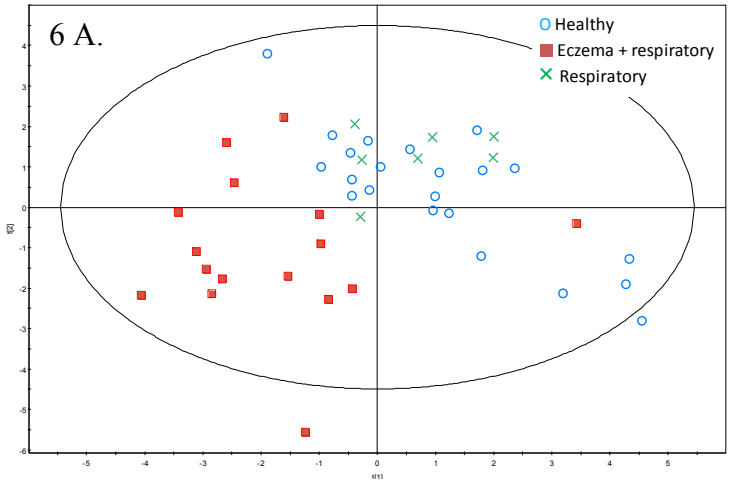
RESULTS AND DISCUSSION

Allergic women have low PUFA levels in breast milk and serum, despite high fish intake

With the aim to investigate whether fatty acid pattern could be related to different allergic manifestations, we analyzed 45 women for the fatty acid pattern in serum and breast milk. These women were either non-allergic (n=22), had respiratory allergy (n=7), had respiratory allergy combined with eczema (n=16) or had eczema alone (n=1). Breast milk was collected one month after delivery of the child. The results were analyzed by the multivariate pattern analysis method PLS-DA in which groups can be separated on the basis of a very large number of variables. We found that women with an allergic phenotype that included eczema had a fatty acid pattern in breast milk and serum that differed significantly from that of women who were non-allergic or had isolated respiratory allergy, Fig. 6.

The two latter groups overlapped almost completely, indicating that they had very similar fatty acid patterns in breast milk and serum. When examining which fatty acids that were responsible for the separation of the women with eczema from the rest, we found that eczematous women had significantly lower levels of several PUFAs in breast milk and serum compared to the other two groups. This included the total level of PUFAs, as well as lower levels of the n-3 PUFAs EPA, DHA, DPA and the n-6 PUFA AA.

The deviating fatty acid patterns of the women with combined respiratory allergy and eczema might depend on the extent of disease. The eczema group included women who suffered from allergy involving two organ systems (skin and airways). They were also sensitized towards a greater number of allergens than the women with isolated respiratory allergy. Another possibility is that partly different types of immune reactions are involved. Thus, in atopic eczema, both Th1, Th2 and $CD8^+$ T cells are involved, while respiratory allergy may be dominated more by Th2-mediated reactions.



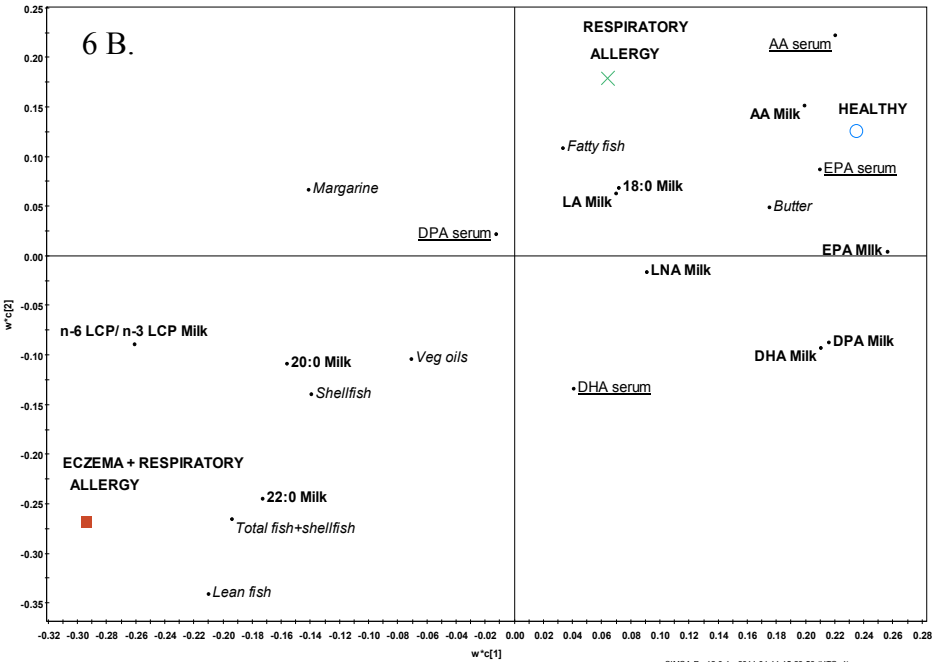


Fig.6 A. *PLS-DA score plot showing an overview of the separation of the three clinical groups: healthy (non-allergic), eczema+respiratory and isolated respiratory allergy.* ***B.*** *PLS-DA loading plot displaying the variables that contributed most to the class separation.*

Regarding the low levels of PUFAs observed in allergic women with respiratory allergy and eczema compared with non-allergic women, several possible mechanisms can be proposed:

1. Low dietary intake of PUFAs may predispose to allergy development. However, when we analyzed dietary data obtained by FFQ, we found that women with extensive allergy including eczema had the highest intake of lean fish, shellfish and total fish intake. Eczematous women thus, had low n-3 PUFA levels despite a high fish intake. Further, the highest intake of fatty fish was seen in women with isolated respiratory allergy, Fig. 6B, suggesting that their "normal" PUFA levels were in fact, lower than would be expected according to their dietary intake.
2. High levels of PUFAs could protect against eczema/extensive allergic disease. Low levels of PUFAs despite high fish intake could be caused by increased levels of exercise in the allergic women. Helge *et al* [122] showed that exercise leads to increased incorporation of EPA and DHA in muscle phospholipids. If other PUFAs are also incorporated in muscle cell membranes and if women with extensive allergy are more physically active than non-allergic women, this could explain the lower amounts of PUFAs in the former group.
3. High levels of PUFAs could confer protection to eczema/extensive allergic disease. Low levels of PUFAs despite high fish intake could depend on a relative

dysfunction of Δ6- and Δ5-desaturases. These enzymes elongate LA and LNA to AA, EPA, DHA and DPA. Several other studies have suggested that enzymatic dysfunction could explain low n-3 PUFA levels in allergic subjects [86, 123, 124].

4. High levels of PUFAs could give protection from eczema/extensive allergic disease. Low PUFA levels despite high fish intake could depend on a relative dysfunction of PUFA uptake in the intestines or reduced transport from serum to breast milk.
5. Low PUFA levels in women with extensive allergic disease could be a result of consumption of PUFAs during the allergic inflammation. AA, EPA and DHA could be consumed for e.g. production of mediators produced during inflammation, incorporated into rapidly dividing cells, or consumed in other processes connected to allergic inflammation.

High PUFA levels in cord blood may be a risk factor for subsequent allergy development

To investigate whether or not PUFAs exert a protective effect on allergy development, we examined PUFA levels in the cord blood of a prospective birth-cohort assembled in Jämtland in 1998. Serum had been stored frozen and we selected three clinical well-defined groups: children who had atopic eczema, but no other type of allergy at 13 years of age, children who had asthma and/or hay fever, but no other type of allergy at 13 years of age, and completely non-allergic children, who were also negative in skin prick tests for all tested potential allergens.

We observed that the cord blood of infants who later developed allergy had *higher* PUFA levels at birth than infants who remained non-allergic. This was true of both n-3 and n-6 PUFAs, e.g. AA (n-6) and DHA (n-3), Fig. 7, as well as most of the examined PUFAs (see paper 2). AA and DHA are two of the most important fatty acids during fetal development. AA is a major constituent of vascular endothelium membranes, while DHA is essential for the development of the brain and retina [38]. Thus, DHA and AA are preferentially transported from the mother to the fetus across the placenta [44].

Our results thus suggested that PUFAs, both of the n-3 and n-6 series, in cord blood were *not protective* against allergy development but were rather associated with a *greater risk* of developing IgE-mediated allergy. This finding was surprising considering the multiplicity of studies in which supplementation with n-3 PUFAs to pregnant and lactating women was used in an attempt to prevent allergy development.

Differences in PUFA levels in the fetus could depend on differences in maternal blood PUFA levels, differences in maternal-fetal transport capacity and/or differences in fetal production of PUFAs. Regarding the first possibility, a constant supply of PUFAs to the fetus is thought to operate so as to ensure PUFA availability during critical periods of fetal development [125]. Theoretically, a genetic variability of both placental lipases and placental fatty acid transport proteins might affect the transfer of fatty acids across the placenta.

Stable isotope studies have demonstrated that premature infants are able to synthesize AA and DHA at an age when they would still developmentally be dependent on the placenta [39]. A difference in the fetal ability to produce PUFAs could thus offer a possible explanation for the presently observed disparity in cord fatty acids.

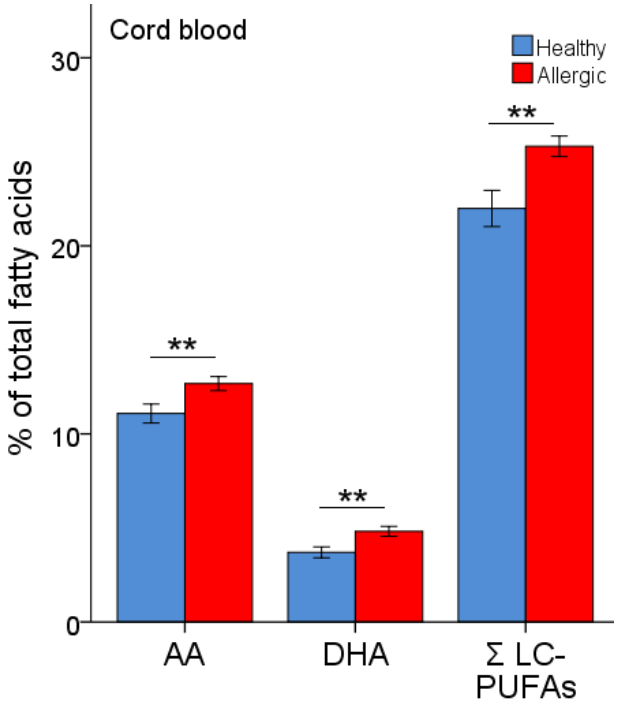


Fig.7 *Fatty acids (% of total fatty acids) in cord serum phospholipids from infants who later developed allergy or stayed non-allergic (**P<0.01, AA= arachidonic acid, DHA= docosahexaenoic acid, LC-PUFAs= long-chain polyunsaturated fatty acids).*

PUFAs in allergic and non-allergic children at 13 years of age

Serum was also obtained and analyzed for PUFA pattern at 13 years of age in the allergic and non-allergic children in the birth cohort. No differences in serum fatty acids were observed between allergic and non-allergic 13-year old adolescents as regards PUFA

pattern. According to the FFQ done at 13 years, the eczema group consumed more low-fat milk, low-fat cheese, sausage and bacon than the non-allergic group. The respiratory group had a higher intake of sausage than the non-allergic controls. Fatty pork products contain much saturated fat. However, the differences in dietary intake were not extensive enough to be reflected as fatty acid differences in serum.

The clear difference in PUFA pattern at birth between the children who later developed allergy or remained non-allergic, and the absence of such a difference between the same groups at 13 years of age, could indicate either that the cord blood concentrations of PUFAs were mainly dependent on maternal factors (dietary intake, PUFA elongation, transplacental transport) while the 13-year serum PUFA pattern naturally depended on the child´s own dietary intake or PUFA elongation. As the allergic phenotype is imprinted very early in life [126], PUFA patterns at birth are likely to be much more important for allergy development than later patterns. Secondly, as suggested in study 1 on allergic women, allergic children may experience enhanced consumption of PUFAs in the body during allergic inflammation. Thus it is not impossible that the allergic children could ingest and/or produce (by elongation) more PUFAs than non-allergic children, but that this does not show up as increased PUFA levels in blood, because PUFAs are consumed during allergy.

Diets rich in PUFAs have different effects in Th1- and Th2-mediated hypersensitivity mouse models

Mouse studies were performed to investigate effects of high dietary intake of PUFAs on DTH and IgE-mediated hypersensitivity reactions. Mice in both models were fed either a control diet (regular mouse chow), or a diet supplemented with fish oil (rich in n-3 PUFAs) or sunflower oil (rich in n-6 PUFAs).

We selected two hypersensitivity models: In the first model, delayed-type hypersensitivity (DTH), known to be exerted by $CD4^+$ T cells was elicited. Immunization with ovalbumin (OVA) in Freund´s complete adjuvant was used to elicit $CD4^+$ T cells. Later injection of OVA into the skin (here: paw) led to accumulation of $CD4^+$ T cells and monocytes within 24-48 h. In the second model, injection of the model antigen OVA together with the adjuvant alum, was performed to induce production of IgE antibodies. Challenge with OVA in the airways led to IgE-mediated hypersensitivity with an accumulation of eosinophils in the airways.

We found that fish oil supplementation reduced some immune activity parameters in the DTH model, foremost proliferation and cytokine production by lymphocytes in draining lymph nodes when stimulated with the recall antigen, OVA, Fig 8 A. Animals that were

fed sunflower oil also showed decreased IFN-γ production, although to a lesser extent than mice fed a diet supplemented with fish oil, Fig 8 A.

In the airway hypersensitivity model, we noted that mice that were given a diet supplemented with fish oil had higher levels of antigen-specific IgE in serum and had a higher proportion of eosinophils in the bronchoalveolar fluid, than mice fed a control diet, Fig. 8 B.

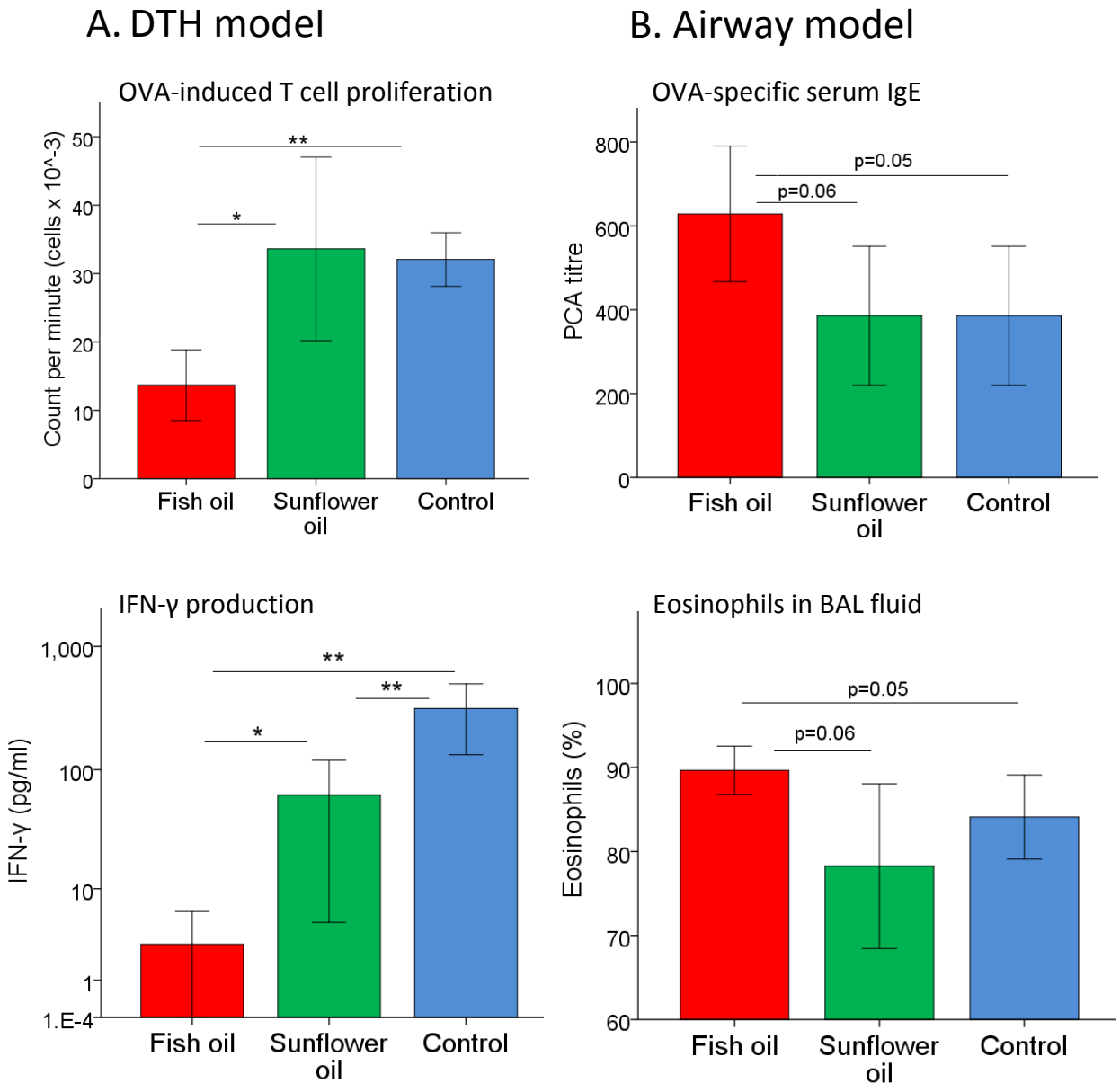


Fig. 8 *Effects on fatty acid supplementation in* ***A.*** *DTH model: Proliferation of cells from draining lymph nodes and proliferation of IFN-γ.* ***B.*** *Airway model: Serum levels of OVA-specific IgE and proportion of eosinophils from bronchoalveolar lavage fluid.*

The results demonstrate that a fish oil diet suppresses effector functions of Th1-type T cells (less production of IFN-γ) while increasing IgE production, which depends on the action of Th2 cells. The reduction of the Th1-reaction could depend on a lower prevalence and/or functional activity of the Th1 memory cells, while we found no evidence for any direct anti-inflammatory effects of fish oil. Thus, the marginally decreased DTH could be explained by a reduced prevalence/functional activity of OVA-specific memory Th1 cells.

The increased IgE production in mice fed fish oil rich in n-3 PUFAs was very interesting and somewhat controversial, as support has been reported for fish oil to reduce allergy [73, 74, 127]. However, there are also many studies that have not found a beneficial effect of fish oil on allergy development [76, 80, 128, 129].

Early feeding of fish is associated with protection against eczema [5-7]. Eczema is a complex condition involving both Th1 and Th2 $CD4^+$ T cells [130], where Th1 cells induce the chronic inflammation reaction with prolonged effect [131]. $CD8^+$ cells are also prevalent in the early phase of the disease [132]. If fish or fish oil is able to reduce Th1-mediated reactions, this might explain why diets including fish could protect against eczema. However, one must also consider the possibility that other components in fish are responsible for the protective effects, such as vitamin D, vitamin E or selenium.

Reduction of PUFAs during the allergic inflammatory reaction

A significant reduction of PUFAs was seen in the animal study during the challenge phase and the resulting allergic inflammatory reaction. The decrease was most obvious in the airway model, and the drop in serum EPA levels during the challenge phase correlated positively with the serum levels of OVA-specific IgE. The quite drastic fall in serum PUFA levels, despite continued intake, indicate a pronounced consumption of long-chain PUFAs during the inflammatory process.

Consumption of PUFAs was much less pronounced in the DTH model. However, footpad swelling correlated positively with reduction in serum EPA levels during the challenge phase. The lesser amount of PUFA consumption could depend on a difference in size of the affected organs; one paw in the DTH-model as compared to the entire respiratory system in the asthma model. A higher consumption of PUFAs in the respiratory model may also depend on eosinphilic involvement, since eosinophils are known to be versatile producers of products from PUFAs. Further, we noted a reduction of PUFAs during the Th2-sensitization phase but not Th1-sensitization phase. This could be due to an enhanced production of lipid mediators that affect the outcome of the interaction between APCs and naïve T cells, leading to Th2 maturation. In accordance, the lipid mediator

PGE_2 that is produced from AA promotes the development of APCs that favor Th2 maturation.

Consumption of PUFAs during allergic reactions would explain the low levels of PUFA in lactating women with an extensive degree of allergy including eczema, compared to non-allergic women and women with isolated respiratory allergy. The group of women with an extensive degree of allergy suffered from both skin and respiratory atopy involving more than one organ and a greater number of allergens compared to the isolated respiratory group. The extensive allergic inflammation would probably consume more PUFAs than the less extensive respiratory disease.

The suggested effect of PUFAs in mouse hypersensitivity models can thus be summarized as:

- PUFAs are consumed during the inflammatory reaction, as a suggestion as substrates for production of inflammatory mediators. Low levels of PUFAs in allergic subjects could thus depend on increased PUFA consumption in these subjects, rather than decreased intake, uptake or production from lipid precursors.
- PUFAs might affect the outcome of the sensitization phase, particularly promoting Th2 sensitization. In contrast, PUFA feeding decreased maturation of functional Th1 cells.
- Concerning decreased maturation of Th1 cells, similar effects were seen with either fish oil, containing a high proportion of n-3 PUFAs, or sunflower oil, containing mainly n-6 PUFAs.

Dietary habits among farmer families could be a protective factor against allergy in the farming environment

A small dairy farm is the most allergy-protective environment observed in economically developed countries. The reason for this is not known, but the diet of farmers and their children may play a role. Since almost all Swedish infants born today are exclusively breast-fed for several months, breast milk is the major dietary factor that could modulate allergy development. We investigated breast milk and serum from women on small dairy farms and non-farming control mothers living in the same rural area with respect to PUFA pattern. We also investigated the diet of the mothers by FFQ and by 24-h recall and 24-h dietary record. Fig 9A shows the composite pattern of breast milk PUFA composition and maternal diet – breast milk PUFAs are shown in italics. Milk from farming women had higher levels of the saturated fatty acids 18:0 and 20:0, while milk

from non-farming women had higher levels of the saturated fatty acid 22:0 and the PUFAs LA (n-6), LNA (n-3) and DPA (n-3), Fig 9B. Dietary habits among farming women also differed significantly in several respects from the habits of non-farming women, Fig 9B. Farmers had a higher intake of foods containing saturated fatty acids, such as butter, whole milk and whole-fat cream. Butter, in particular, is rich in the fatty acid 18:0. In contrast, non-farming women consumed more margarine, which contains high levels of PUFAs such as LA (n-6) as well as a substantial amount of LNA (n-3). Non-farming women also consumed more low-fat milk and cream.

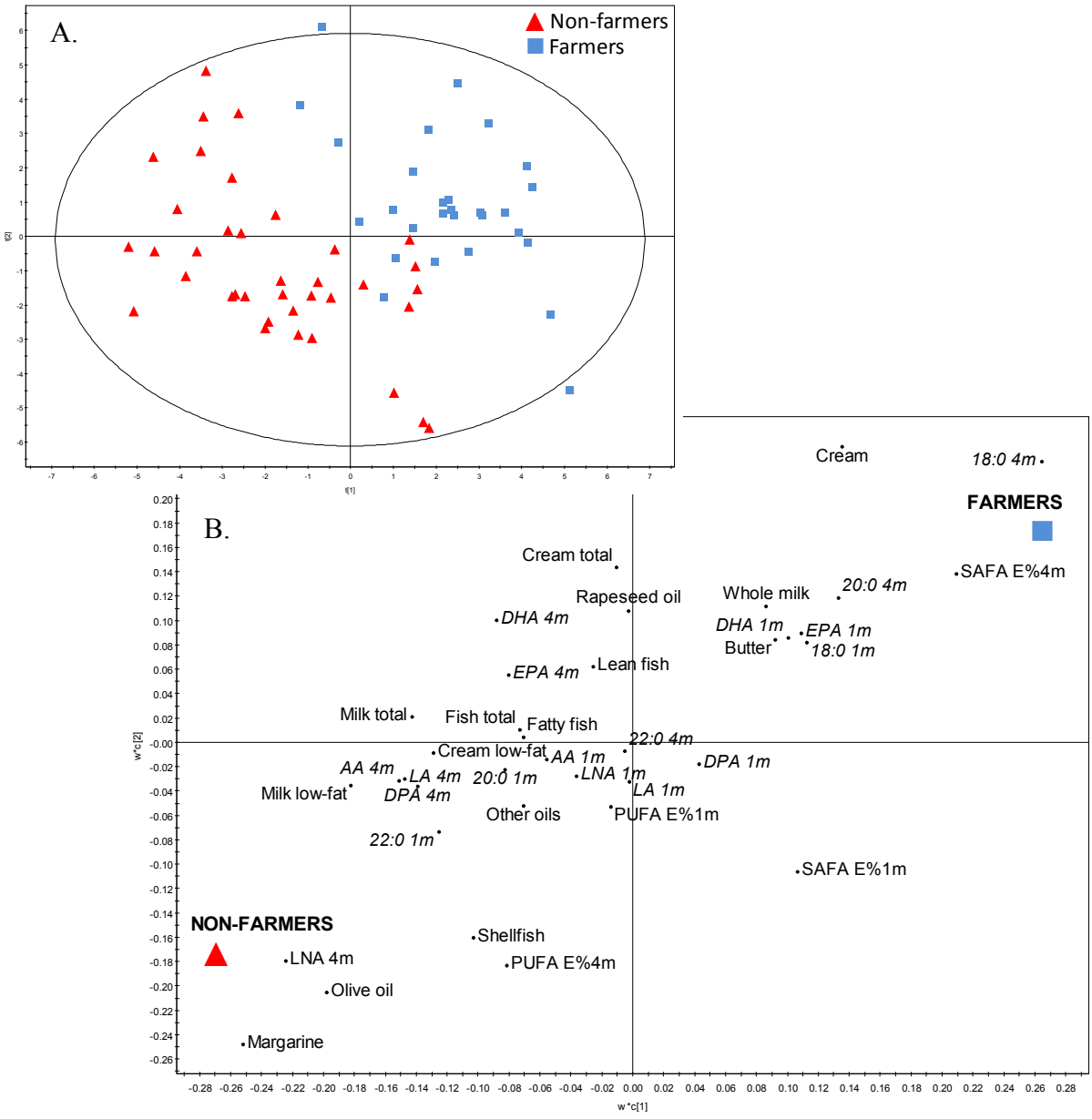


Fig.9 A. *PLS-DA score plot showing the separation between the groups of mother-infant pairs; farmers and non-farming controls.* ***B.*** *PLS-DA loading plot displaying the variables that contributed most to the separation between farmers and controls.*

Children born in dairy farming families have a low incidence of allergies. Our results suggest that intake of butter, rather than margarine, could be one explanation for the allergy-preventive lifestyle at the small family farm. Several studies have shown that intake of PUFA-rich margarine is associated with a higher prevalence of allergies, while intake of butter is related to a lower incidence of allergies [58-62]. There can only be speculation of the possible immunomodulatory mechanisms of SAFAs versus PUFAs. However, one explanation could be that SAFAs are relatively inert and are perhaps not substantially active in immune reactions. PUFAs are more reactive because of the double bonds and their flexibility and folding abilities. They affect gene transcription, membrane fluidity and thrombocyte activation and coagulation, which may all affect immune responses. PUFAs are also precursors to several inflammatory mediators such as prostaglandins, leukotrienes and thromboxanes.

Farmers seem to have more traditional dietary habits than non-farmers when it comes to fats and dairy products. It is probably natural for farmers to eat unprocessed products from their own production. Even if they do not produce foods such as cream and butter themselves, they obviously choose to buy similar natural products. Although the control group lived in the same rural areas as the farmers, they were evidently more prone to adopt a more "modern" diet regarding margarine and low-fat products.
It is impossible to draw conclusions in the present study concerning the effect of the individual infant´s breast milk PUFA consumption and subsequent allergy development, since the clinical data of the children included have not yet been evaluated.

High margarine intake is associated with allergy

We compared the consumption of butter versus margarine in the three cohorts investigated, Fig 10.
Among the lactating women in cohort I, we observed a significantly higher butter intake in non-allergic women than in women with isolated respiratory allergy. Comparing all allergic women (including those with eczema and respiratory allergy) with the non-allergic group, a significantly higher intake of butter among non-allergic women was found, as well as a trend toward higher margarine consumption among allergic women.
In cohort II, dietary data were available from 13-year-old adolescents. The results for butter and margarine consumption did not differ greatly between allergic and non-allergic adolescents. However, a tendency toward higher margarine and butter intake was reported in the allergic group.

As mentioned above, there were obvious differences in butter and margarine consumption between farming and non-farming women. Farmers ate significantly more butter and non-farmers ate more margarine.

The dietary patterns in all three cohorts (I. Allergic and non-allergic women, II: Allergic and non-allergic 13 year olds´ and III. Farming and non-farming women) indicated strong associations both between high margarine intake and high incidence of allergy and between a high butter intake and a low prevalence of allergy.

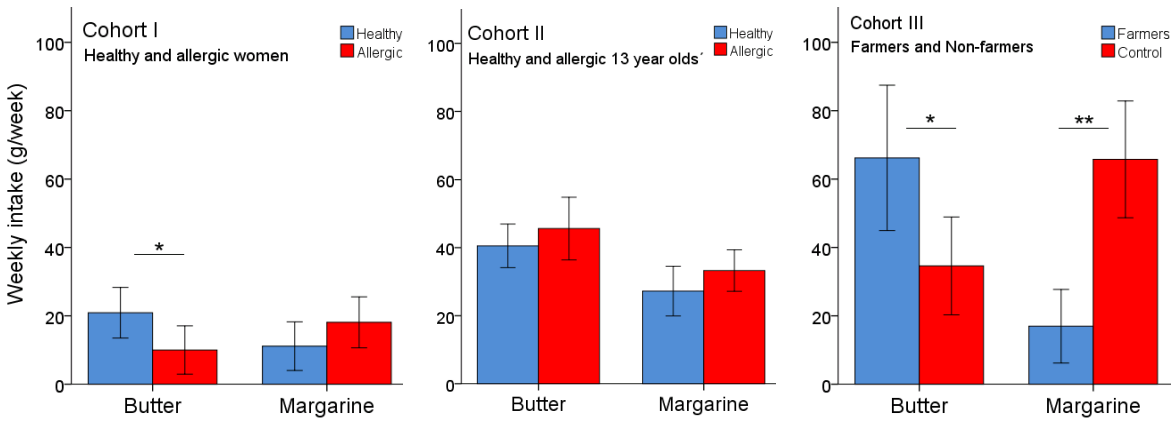


Fig. 10 *Butter and margarine intake according to FFQ in the cohorts investigated in the present thesis; Cohort I – Allergic and non-allergic lactating women, Cohort II – Allergic and non-allergic 13-year-old adolescents and Cohort III – Farming and non-farming women. Only butter and margarine as bread spread was asked for in cohort I, and this does therefore not include use in cooking.*

CONCLUSIONS

- PUFAs appear to facilitate atopic sensitization and allergy development. This is true of n-3 PUFAs, as opposed to the previous models that attribute an allergy-preventive effect. Thus, mice fed a diet rich in n-3 PUFA produced more IgE than mice fed a regular diet (low in fat). Further, a high concentration of PUFAs, both of the n-3 and n-6 series, in the newborn infants (umbilical cord blood) was associated with a high risk of allergy development.

- PUFAs are consumed in the body during the inflammation reaction. This may explain the reduced breast milk and serum levels of PUFAs in allergic subjects, despite a similar, or even higher, intake of foods containing PUFAs. For example, women with allergy including eczema have lower breast milk and serum levels of typical fish PUFAs compared to non-allergic women and women with respiratory allergy, despite a high intake of fish.

- Women on small dairy farms had a breast milk fatty acid pattern that differed from non-farming women in the same rural region, most notably showing higher levels of the saturated fatty acid 18:0. Their dietary intake of butter and dairy products rich in saturated fat was also high, while controls ingested relatively more margarine containing n-6 PUFAs and had higher levels of several PUFAs in their breast milk. These differences in diet will, most likely, later also be manifest in children growing up on farms and in their non-farming counterparts. On the basis of our findings of PUFAs as a risk factor for sensitization and allergy, we propose that a diet containing less PUFAs may be one factor that helps to explain the allergy-preventing effect of growing up on a dairy farm.

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