From
the Department of Paediatrics at the Dr. von Hauner Children's Hospital,
Ludwig-Maximilians-University of Munich, Germany
Director: Prof. Dr. med. Dietrich Reinhardt
and
the Institute of Epidemiology, Helmholtz Zentrum München,
German Research Center for Environmental Health, Neuherberg, Germany
Director: Prof. Dr. Dr. H.-Erich Wichmann

Socioeconomic determinants of dietary fat intake and the effect of dietary fat intake on allergic diseases in children

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by
Stefanie Sausenthaler
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Supervisor/Examiner:	Prof. Dr. B. Koletzko
Co-Examiners:	Prof. Dr. R. von Kries Prof. Dr. P. Thomas
Co-Supervisor:	Dr. J. Heinrich
Dean:	Prof. Dr. med. D. Reinhardt
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CONTENTS

LIST OF ABBREVIATIONS

CI	Confidence interval
COX	Cyclooxygenase
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FFQ	Food-frequency questionnaire
HETE	Hydroxyeicosatetraenoic acid
HPETE	Hydroperoxyeicosatetraenoic acid
IFN	Interferon
IgE	Immunoglobulin E
IL	Interleukin
ISAAC	International Study of Asthma and Allergies in Childhood
LISA	Influences of Lifestyle Related Factors on the Immune System and the Development of Allergies in Childhood
LOX	Lipoxygenase
LT	Leukotriene
MUFA	Monounsaturated fatty acid
OR	Odds ratio
PG	Prostaglandin
PUFA	Polyunsaturated fatty acid
Q	Quintile
SAS	Statistical Analysis System
SD	Standard deviation
SPT	Skin prick test
T	Tertile
Th2	Type 2 T helper cell
Th1	Type 1 T helper cell
TNF	Tumor necrosis factor
TX	Thromboxane

1 INTRODUCTION

There is a wide consent that the prevalence of allergic diseases has increased over the past decades in countries with a western lifestyle. This increase has been paralleled by dietary changes, in particular the increased intake of foods rich in n-6 polyunsaturated fatty acids (PUFA) and the reduced intake of foods rich in n-3 PUFAs (1). Due to the proinflammatory properties of n-6 PUFAs and the less inflammatory effects of n-3 PUFAs, a proportionally high intake of n-6 PUFA has been linked to an increased risk for allergic diseases, whereas a high n-3 PUFA intake has been suggested to have protective effects.

1.1 Allergic diseases and allergic sensitisation

1.1.1 Definition of allergy, hypersensitivity and atopy

The word *allergy* derives from the Greek words *allos* ("other") and *ergon* ("work") and was coined by the Austrian paediatrician Clemens von Pirquet in 1906. He originally used the term to describe an exaggerated biological reactivity to foreign substances (2;3). Today, the term allergy is used to mean a hypersensitivity reaction initiated by specific immunological mechanisms. Hypersensitivity describes objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons (4).

Hypersensitivity reactions are classified as immediate or delayed (type I – IV) according to the classification scheme originally designed by Gell and Coombs in 1963 (5). Type I allergies are mediated by immunoglobulin E (IgE) antibodies. First allergen contact leads to the production of IgE that binds to the high-affinity receptors on mast cells and basophils. Repeated exposure to the particular allergen leads to cross linking of receptor-bound IgE antibodies, which initiates mast cell degranulation and subsequent release of histamine, prostaglandins, leukotrienes and other mediators. This early reaction can be followed some hours afterwards with a late response, in which immune cells infiltrate into the skin. These processes evoke an inflammatory response responsible for the typical symptoms of allergic diseases, such as asthma, allergic rhinoconjunctivitis and atopic eczema (6). Depending on the individual and the type of allergen, the symptoms can be either systemic or local. However, up to now the reason why hypersensitivity to the same allergen can cause different clinical manifestations is poorly understood.

In general, the genetic predisposition to produce IgE antibodies in response to exposure to commonly occurring allergens is described by the term *atopy* (from the Greek *atopos*,

meaning “out of place”). Consequently, atopic subjects can develop typical symptoms of asthma, rhinoconjunctivitis and eczema (4). Allergic sensitisation, which describes the presence of elevated serum levels of allergen-specific IgE antibodies, is thought fundamental to these disorders and is therefore considered as potential risk factor for the development of allergic diseases (7-9).

1.1.2 Prevalence of allergic diseases in childhood

The prevalence of allergic diseases among children and adults has increased worldwide over the past decades (10-14). However, there is a striking geographic variation in the prevalence of allergic diseases between and even within countries (15;16). The highest rates of symptomatic asthma prevalence in childhood have been found in the United Kingdom, New Zealand, Australia and the Republic of Ireland, followed by countries in North, Central and South America. The lowest prevalence rates were observed in Eastern European countries, Indonesia, Greece, China, Taiwan, Uzbekistan, India and Ethiopia. In these countries, the prevalence rates for allergic rhinoconjunctivitis and eczema were also the lowest, but the highest prevalence rates for allergic rhinoconjunctivitis and eczema were reported from countries all over the world. (16).

In Germany, the fall of the Berlin wall offered a new opportunity to study potential causes of regional differences in genetically similar populations. Shortly after reunification, lower prevalence rates of asthma, hay fever and atopic sensitisation have been reported both among children and adults in the East compared to the West of Germany (17;18). About five years after reunification, the prevalence of allergic diseases had significantly increased in Eastern Germany (14;19) indicating a tendency to converge with the western prevalence (20). This assumption is now confirmed by results from the National Health Interview and Examination Survey for Children and Adolescents (KiGGS) that showed no differences in the prevalence of asthma, hay fever and atopic dermatitis between East and West Germany anymore (21). Since dramatic changes towards western conditions have accompanied the reunification in the Eastern part of Germany, this gave indirect support for the hypothesis that western lifestyle might be associated with allergic diseases (14;22). Recent data from the International Study of Asthma and Allergies in Childhood (ISAAC) (Phase Three, 2002-2003) indicate symptom prevalence rates of 12.8% for asthma, 6.9% for allergic rhinoconjunctivitis and 7.9% for eczema in 6-7 year old children living in Germany (10).

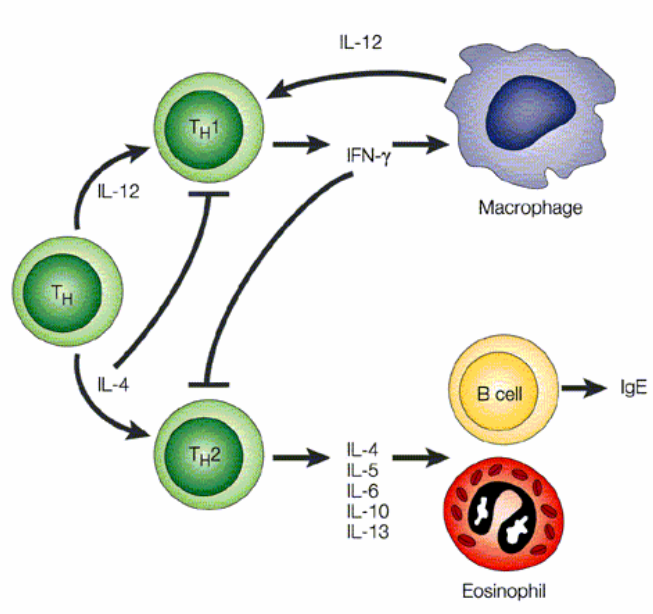
In addition to these geographic differences, the prevalence of allergic diseases also varies by age. The natural history of atopic manifestations is characterised by a typical sequence of

clinical symptoms, with some symptoms becoming more prominent while others subside. In general, eczema is most often the first manifestation of the atopic triad (23), which is then replaced by rhinoconjunctivitis and asthma (24-26). Similarly, allergic sensitisation in infancy occurs predominantly to foods, but in later childhood sensitisation to inhalant allergens is more prevalent (27).

1.1.3 Immune mechanisms in the pathogenesis of allergic diseases

The recent concept of allergic reactions suggests that allergen-reactive type 2 T helper cells (Th2) play a triggering role in the allergic inflammatory cascade. Activated Th2 lymphocytes produce certain cytokines, which are responsible for eosinophil activation and IgE production necessary for allergic inflammation (28). According to the so-called hygiene hypothesis, changes in the exposure to infectious substances and microbial products associated with a cleaner indoor environment result in skewing of T cell responses towards the Th2 cytokine profile (29;30). Th1 (Type 1 T helper cell) and Th2 subsets develop from the same precursor, which are naïve T lymphocytes (figure 1).

Figure 1 T-helper cell differentiation and production of cytokines (adapted from O'Shea et al 2002 (31))



Th1 cells differentiate in response to microbial stimulation of antigen-presenting cells under the influence of interleukin (IL)-12. They secrete interferon (IFN)-γ, which is important in macrophage activation and downregulates the proliferation of Th2 cells. This indicates that Th1 immune responses are highly protective against infections, especially against intracellular organisms (31;32). In contrast, Th2 differentiation occurs in response to environmental

allergens under the influence of IL-4. Activated Th2 lymphocytes produce IL-4, IL-5, IL-6, IL-10 and IL-13, which promote the production of IgE antibodies by B cells and induce the activation of eosinophils. In addition, IL-4 antagonises Th1 differentiation.

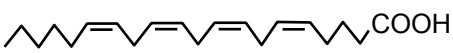
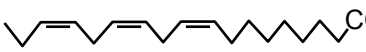
Although the hygiene hypothesis is generally accepted as the most reasonable explanation for allergy epidemics, its immunological basis is a matter of controversy. More recently, the potential role of a third set of cytokines called T-regulatory cells has been emphasised (33) and let assume that the mechanisms involved in allergic reactions might be more complex.

1.2 Dietary fat and polyunsaturated fatty acids

1.2.1 Biochemistry of fatty acids

Fatty acids are composed of a hydrocarbon chain with a methyl group and a terminal carboxyl group. The most abundant fatty acids have straight chains of an even number of carbon atoms, which vary in length from four to 24 carbon atoms or more. They further may be saturated or unsaturated depending on the existence of 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). Among unsaturated fatty acids, distinction between two different geometric configurations, denoted as *cis* and *trans* configuration, can be made. While *cis* isomers have both hydrogen atoms of the double bond on the same side of the molecule, *trans* isomers have it on the opposite side of the molecule (34;35). PUFAs usually have 18 to 22 carbon atoms and are categorised according to the position of the last double bond near their methyl end mainly into those of the n-6 and the n-3 series. Table 1 shows the nomenclature of PUFAs that are important in human nutrition.

Table 1 Nomenclature of n-6 and n-3 polyunsaturated fatty acids

Trivial name	Short designation	Structural formula
n-6 PUFA		
Linoleic acid	18:2n-6	
Arachidonic acid	20:4n-6	
n-3 PUFA		
α-Linolenic acid	18:3n-3	
Eicosapentaenoic acid	20:5n-3	
Docosahexaenoic acid	22:6n-3	

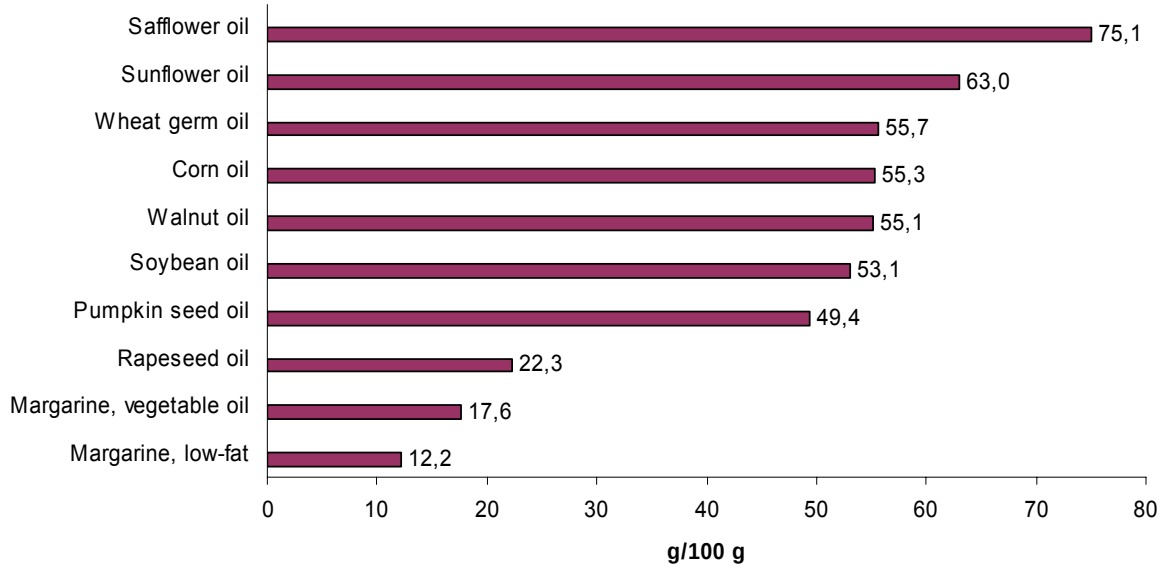
Linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are considered essential fatty acids because they cannot be synthesised *de novo* by mammals and must be consequently obtained from the diet. They can be metabolised into their long-chain derivates arachidonic acid (20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3).

1.2.2 Dietary sources of polyunsaturated fatty acids

N-6 polyunsaturated fatty acids

Linoleic acid is the major dietary n-6 PUFA mainly found in vegetable oils, such as oils made from safflower, sunflower, wheat germ, corn, walnut, soybean, pumpkin seed, and rapeseed (36). In Germany, margarine contains 80% fat, which is mainly derived from vegetable oils, especially sunflower, safflower and rapeseed oil. The German Research Institute for Food Chemistry (Deutsche Forschungsanstalt für Lebensmittelchemie) indicates an average linoleic acid content of 17.6g/100g for vegetable oil margarine and 12.2g/100g for low-fat margarine (figure 2).

Figure 2 Food sources of linoleic acid (36)

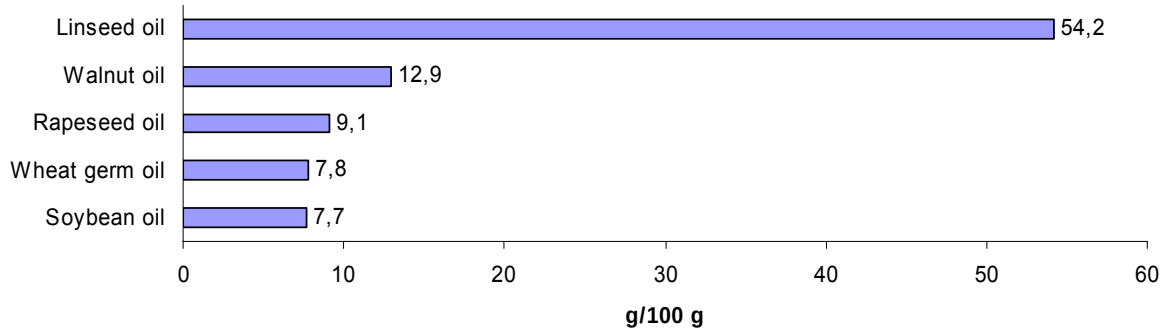


In contrast, arachidonic acid contributes less than 2% (37) to the total dietary n-6 PUFA intake. It is only found in animal sources, such as meat, poultry, fish and eggs since plants do not have the capability to convert linoleic acid to arachidonic acid.

N-3 polyunsaturated fatty acids

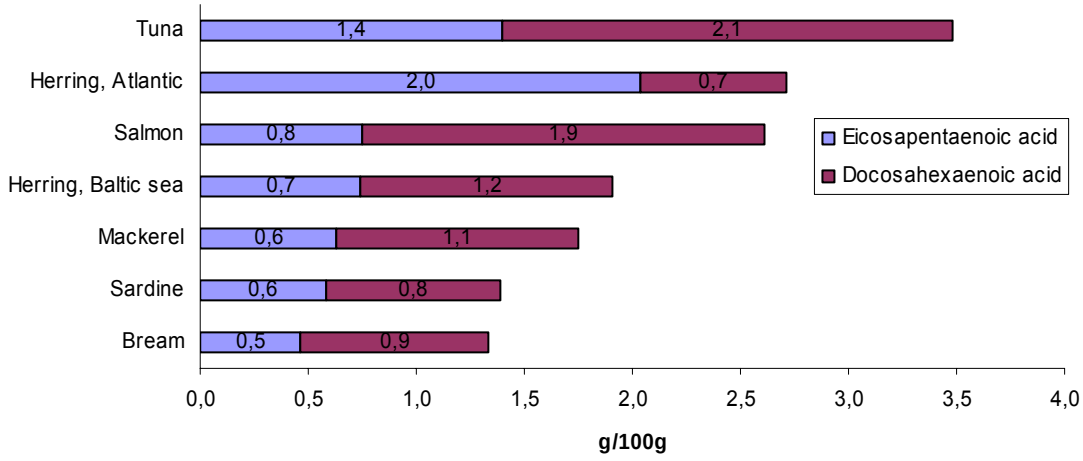
α-Linolenic acid contributes about more than 80% to total dietary n-3 PUFA intake in Germany, while EPA and DHA intake only play a minor role (37). Although small amounts of α-linolenic acid are ubiquitous in foods, linseed oil is probably the richest source of α-linolenic acid containing about 55g/100g. Other vegetable oils with relatively high amounts of α-linolenic acid are walnut, rapeseed, wheat germ and soybean oil (figure 3).

Figure 3 Food sources of α-linolenic acid (36)



EPA and DHA are mainly derived from oily fish, which are mainly sea-fish. In a German diet, they contribute less than 20% to the total n-3 PUFA intake (37). Some fish that are rich in EPA and DHA are shown in figure 4.

Figure 4 Food sources of eicosapentaenoic and docosahexaenoic acid (36)



In the year 2000, about 75% of fish consumed in Germany was sea-fish with Alaska Pollock (28.5%), herring (18.5%) and tuna (13.2%) followed by salmon (6.0%) holding the highest market shares (38).

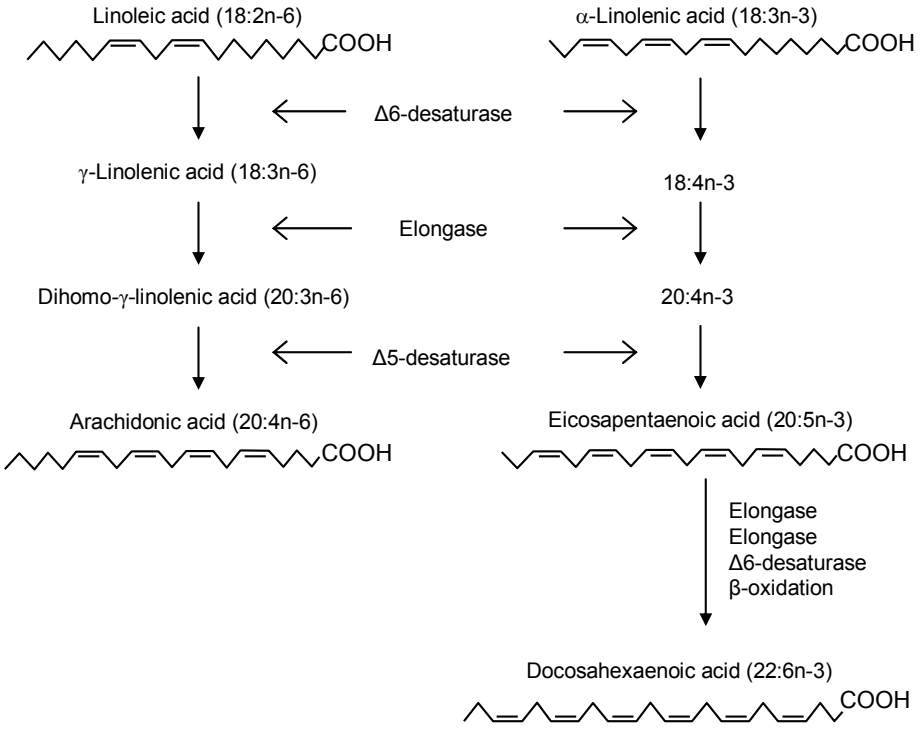
To estimate the contribution of these food sources to the total intake of n-6 and n-3 PUFAs, quantitative information on food consumption must be obtained.

1.2.3 Metabolic pathway of polyunsaturated fatty acids

Linoleic acid and α-linolenic acid can be metabolised by introduction of double bonds (desaturation) and by lengthening the hydrocarbon chain (elongation) into their long-chain derivates arachidonic acid, EPA and DHA. The enzymes necessary for these sequential activities are mainly found in the endoplasmic reticulum of liver cells (39;40).

Linoleic acid is initially metabolised by the enzyme Δ6-desaturase to γ-linolenic acid (18:3n-6), followed by an elongation to dihomo-γ-linolenic acid (20:3n-6), and is subsequently transformed into arachidonic acid, catalysed by the enzyme Δ5-desaturase (figure 5).

Figure 5 Metabolism of n-6 and n-3 polyunsaturated fatty acids (adapted from Calder 2005 (39))



Using the same enzymatic pathway as n-6 PUFAs, α-linolenic acid can be converted to the long-chain fatty acid EPA. Further conversion of EPA into DHA involves the addition of two carbon atoms to form docosapentaenoic acid (22:5n-3), the addition of two more carbons, desaturation and removal of two carbons by β-oxidation. Arachidonic acid can also be metabolised by the same series of enzymes (41).

As this pathway illustrates, the members of the n-6 and n-3 fatty acid families are not convertible, but compete for several enzymes in their metabolism. Although the preferred substrate for Δ6-desaturase is α-linolenic acid, the excess of linoleic acid typical of human diets leads to a greater net conversion of linoleic acid compared with α-linolenic acid (39). Furthermore, the conversion rate of α-linolenic acid to long-chain n-3 PUFAs is limited (42;43). However, the competition for the same enzymes led to the idea that the ratio of n-6 PUFA to n-3 PUFA in the diet is of particular importance.

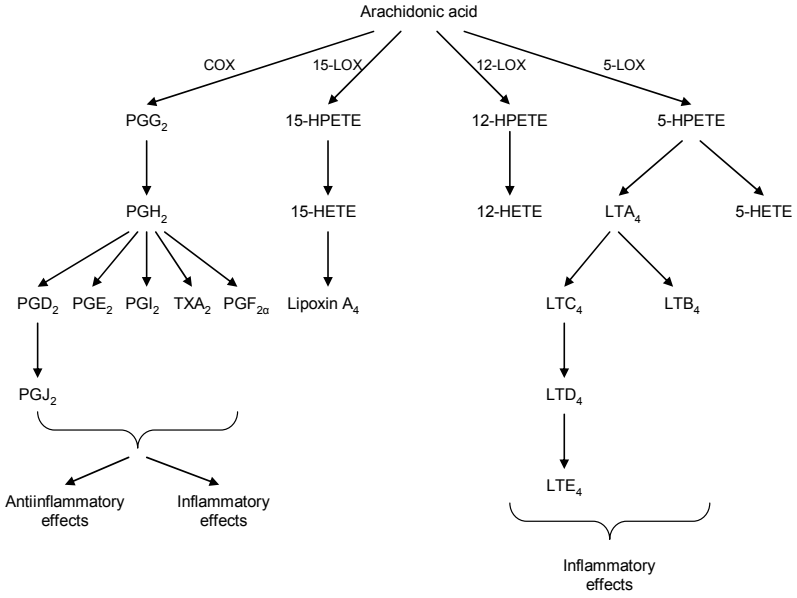
1.3 Eicosanoids and allergic inflammation

The first step of eicosanoid biosynthesis is the release of 20-carbon PUFAs from membrane phospholipids by the enzyme phospholipase A_2. Eicosanoids include prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs) and other oxidised derivates. Because inflammatory cells typically contain high proportions of arachidonic acid and low proportions of n-3 PUFAs, arachidonic acid is the principal precursor for eicosanoid synthesis (44).

Arachidonic acid derived eicosanoids

Free arachidonic acid can act as substrate for different enzymes. Metabolism by cyclooxygenase enzymes (COX 1 and COX 2) gives rise to the 2-series PGs and TXs, while lipoxygenase enzymes (LOX) produce LTs and related compounds (figure 6). The 5-LOX pathway provides 5-hydroperoxyeicosatetraenoic acid (HPETE), 5-hydroxyeicosatetraenoic acid (HETE) and the 4-series LTs.

Figure 6 Pathway for the conversion of arachidonic acid to eicosanoids (44)

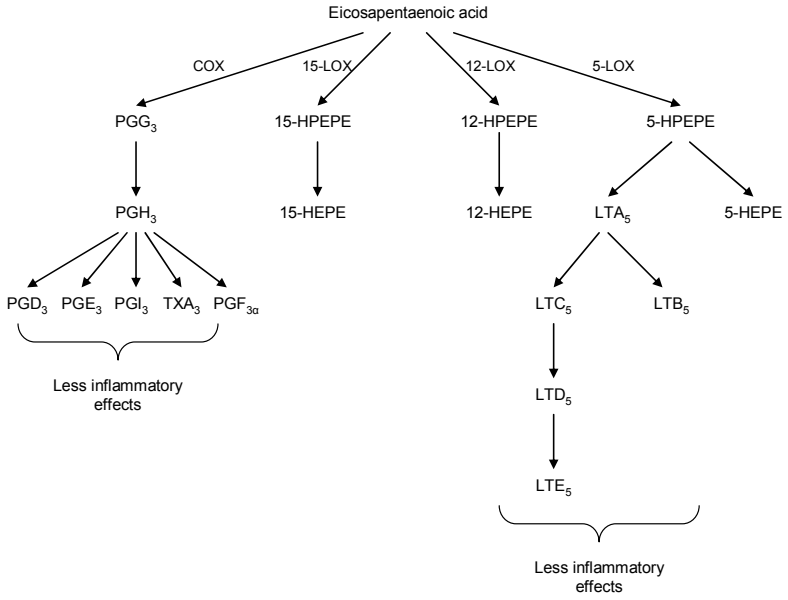


Of particular relevance in the context of allergic diseases is the ability of PGE_2 to inhibit the production of anti-inflammatory Th1 cytokines IL-2 and IFN-γ. PGE_2 also induces COX 2 and so up-regulates its own production and induces the production of IL-6 by macrophages. As it does not affect the formation of inflammatory Th2 cytokines IL-4 and IL-5, allergic inflammation might be promoted by shifting the balance between Th1 and Th2 cells (45). It further has been postulated that PGE_2 stimulates B-lymphocytes to produce IgE (46). Apart from these proinflammatory effects, PGE_2 has also been shown to inhibit 5-LOX and so decreases the production of proinflammatory 4-series LTs and induces 15-LOX, thus promoting the formation of lipoxins that have been found to have anti-inflammatory effects. Thus, PGE_2 possesses both pro- and anti-inflammatory actions. In addition, LTB_4 is proinflammatory and enhances inflammation by increasing vascular permeability. It is a potent chemotactic agent for leucocytes, induces the release of lysosomal enzymes, and promotes the production of inflammatory cytokines TNF (tumor necrosis factor) α, IL-1 and IL-6 by macrophages (47;48). LTC_4, LTD_4 and LTE_4, also known as slow reacting substances, are believed to be the major mediators of asthmatic bronchoconstriction. They stimulate airway smooth muscle cells, mediate vasoconstriction and mucus secretion (45).

N-3 PUFA derived eicosanoids

EPA also acts as substrate for COX and LOX enzymes, giving rise to a different family of eicosanoids: the 3-series PGs and TXs, the 5-series LTs and hydroxy-EPAs (figure 7). Thus, less substrate is available for synthesis of eicosanoids from arachidonic acid and mediators formed from EPA are considered to be less potent compared with those formed from arachidonic acid. This antagonistic action is described as the key anti-inflammatory effect of n-3 PUFAs (44).

Figure 7 Pathway for the conversion of eicosapentaenoic acid to eicosanoids (44)



In addition to altered eicosanoid production, it has been proposed that n-3 PUFAs alter the expression of inflammatory genes by inhibiting transcription factors, such as nuclear factor κB (NF-κB) (39). Furthermore, novel groups of mediators, termed resolvins, docosatrienes and neuroprotectins, which are formed from EPA and DHA and which are supposed to have anti-inflammatory properties, have recently been identified (49;50).

1.4 State of the art of dietary fat intake and allergic diseases

Several epidemiological studies have analysed the association between dietary fat intake and allergic diseases during the past. These studies were addressing this issue by either estimating dietary fatty acid intake from diet or using food items as surrogate markers for fatty acid intake. Interventional studies have investigated therapeutic and preventive effects of n-3 PUFA supplementation on allergic manifestations. A short summary on the main findings is given below, while a more extensive literature review has been published in Sausenthaler S, Koletzko B, Heinrich J. *Dietary fat intake and allergic diseases.* Current Nutrition & Food Science 2006; 2: 351-359.

Dietary fatty acid intake and allergic diseases

Total PUFA intake has been reported to be positively related to allergic sensitisation in men (51), seasonal allergic rhinoconjunctivitis in women (52), wheeze (53) and asthma (54) in children. Given that total PUFA intake is mainly due to n-6 PUFA consumption in a modern diet (55), these observations are consistent with the findings by Wakai et al (52) suggesting a positive association between n-6 PUFA intake and allergic rhinoconjunctivitis in a Japanese

female population. Some other studies related the ratio of n-6 PUFA to n-3 PUFA in the diet to the prevalence of allergic diseases. An Australian nested case-control study reported an increased risk for childhood asthma within subjects having a high dietary n-6/n-3 ratio compared to those with a low ratio (56). Further evidence has been published by Trak-Fellermeier et al (57) who suggested that a high n-6/n-3 ratio increases the risk for atopic eczema in women and hay fever in men. However, other studies did not detect any association between either n-6 PUFA intake or the ratio of n-6 to n-3 PUFA and allergic outcomes (58-62).

In turn, dietary n-3 PUFA intake has been reported to be protective against allergic diseases. A German cross-sectional study detected that α-linolenic acid intake is associated with a decreased risk for allergic sensitisation and allergic rhinitis in 13-80-year-old subjects (60). Inverse associations have also been described between α-linolenic acid and atopic eczema (57) and between EPA and hay fever (63), respectively. Conflicting results have been published by Broadfield et al (58) who observed a higher intake of EPA and DHA in asthmatic compared ton non-asthmatic subjects.

Food intake and allergic diseases

Fish intake has been reported to be protective against asthma (64;65), allergic rhinitis (66), and atopic diseases (67) in children. Recently, Andreasyan et al (68) analysed the association of dietary fish intake with allergen-specific sensitisation and atopic diseases in allergen-specific subgroups in a cross-sectional study of Australian children at age 8 years. Fish consumption significantly decreased the risk for ryegrass-pure sensitisation, but not house dust mite sensitisation. In a subset analysis, fish consumption was protective against asthma, but only in children with ryegrass-pure sensitisation. Other observational studies did not detect any association for fish intake either in children (69), adolescents (70) or adults (61;71;72).

Margarine intake has been positively linked to an increased risk for allergic sensitisation and atopic diseases (14;67;73) in children. Nagel et al (61) also detected a positive association between margarine intake and the onset of asthma, with a stronger effect in men than in women. These gender-specific differences have already been reported before in children (73). In Germany, Trak-Fellermeier et al (57) observed that in 20-64 year old men, margarine intake was positively associated with hay fever prevalence. They found no associations for allergic sensitisation or atopic eczema. Another large cross-sectional study conducted in Germany (National Health Survey 1998-1999) (74) compared 18-79 year old subjects with

respect to the type of fat they used as spread. In the youngest age group (18-29 years), frequent intake of margarine of any kind was positively associated with current asthma. Through further differentiation by low-fat or regular type of spread, only low-fat margarine and the combination of low-fat margarine and low-fat butter were significant dietary risk factors for current asthma. No associations were found for hay fever, atopic dermatitis or atopic sensitisation against inhalant allergens. Butter intake was also an object of investigation in several studies. Butter intake was reported to have a protective effect on atopy in most studies (14;67;72;75), but not in all (57;61;65;76) and no study reported adverse effects of butter intake on allergic diseases.

Interventional studies

A number of clinical studies have investigated the effect of supplementation with essential fatty acids on atopic dermatitis, predominantly linoleic acid or γ-linolenic acid. The pooled effect size of these findings was recently calculated by a meta-analysis, which did not confirm that supplementation with γ-linolenic has a large effect on the severity of atopic dermatitis (77). The effects of n-3 PUFA supplementation on atopic eczema in turn have been studied much more scarcely. One study reported no beneficial effect of n-3 PUFA combined with n-6 PUFA supplementation on atopic dermatitis (78). In contrast, two other investigations found a significant improvement of the clinical condition after fish oil supplementation (79;80), but one of these trials also found a significant improvement in the control group.

At present, the possible preventive effect of essential fatty acid supplementation on the development of atopic diseases in subjects at high risk of atopy is attracting more attention than therapeutic effects in established atopic diseases. Thus, a double-blind placebo-controlled trial investigated whether dietary supplementation with γ-linolenic acid in infants with maternal history of atopic diseases protects against the development of atopy. Supplementation with γ-linolenic acid for the first 6 months of life was not associated with a significantly reduced prevalence of atopic dermatitis or with lower serum IgE concentrations at 1 year. However, in infants who received supplements, atopic dermatitis, if occurring, seemed to be less severe than in infants in the control group (81). One study investigated the effects of fish oil supplementation on seasonal hay fever but did not find a preventive effect (82).

Several clinical investigations examined the impact of n-3 PUFA supplementation, mainly fish oil, on asthma. A systematic review of randomised controlled trials concluded that there is little evidence to recommend a high dietary intake of marine n-3 fatty acids for asthmatic

patients (83). Recently, it was shown that dietary intervention of n-3 PUFA supplementation and n-6 PUFA restriction from the age of 6 months on significantly reduced the risk of atopic cough at age 3 years (84). Supplementation with perilla seed oil improved ventilatory parameters, but only in a subgroup of asthmatic subjects with suppressed leukotriene C4 production after dietary intervention (85).

In summary, it is still not clear, whether dietary fat intake has an impact on the development of allergic diseases. Although some evidence exists for a protective effect of fish intake and an adverse effect of margarine intake on allergies, and biologically plausible mechanisms have been proposed, results are conflicting.

1.5 Objective of the thesis

As described above, some literature exists on the association between dietary fat intake and asthma, hay fever, eczema and allergic sensitisation. However, previous studies mainly focused on later childhood or adulthood and have mostly underlying a cross-sectional study design. It has been proposed that the immune system develops during very early life. This highlights the need to assess the effect of dietary fat intake during infancy and the exposure to dietary fat during foetal life on the development of allergic diseases. As allergic diseases are known to highly depend on social status, regional and socioeconomic differences in dietary fat intake should also be considered.

Therefore, the main objectives of this thesis are:

I. To describe dietary fat intake in 2-year-old children in relation to region and socioeconomic determinants.

II. To analyse whether maternal dietary fat intake during the last 4 weeks of pregnancy is associated with eczema and allergic sensitisation in the offspring at 2 years of age.

III. To analyse whether dietary fat intake in children is associated with eczema and allergic sensitisation at 2 years of age.

2 MATERIAL AND METHODS

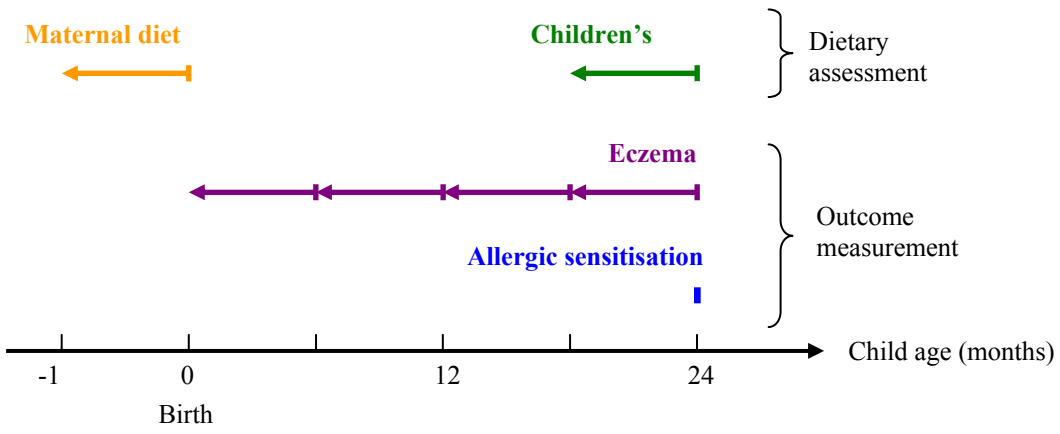
To answer the three key research questions of this thesis, data from a multicentre prospective birth cohort study, the LISA-study, was used.

2.1 Study design and study population

The LISA-study is a prospective birth cohort study, which was originally designed to assess 'Influences of **L**ifestyle related Factors on the **I**mmune **S**ystem and the Development of **A**llergies in Childhood'. Between November 1997 and January 1999, newborns were recruited from 14 obstetrical clinics in the four German cities Munich, Leipzig, Wesel, and Bad Honnef. The target population was restricted to newborns from parents who were born in Germany and had German nationality. In addition, a written informed consent of the parents was required. Exclusion criteria were defined as preterm birth (maturity < 37 gestational week), low birth weight (< 2500 g), congenital malformation, symptomatic neonatal infection, antibiotic medication, hospitalisation or intensive medical care during neonatal period, chronic or immune-related diseases of the mother, maternal long-term medication or abuse of drugs or alcohol. Thus, a total of 3097 newborns were enrolled in the study.

Shortly after birth, questionnaires on family history of atopy, socioeconomic status, smoking during pregnancy, and maternal diet during pregnancy were administered to the parents. Data on children's health, diet, and different lifestyle factors were collected by using repeated parental-completed questionnaires at regular time intervals during the follow-up period of 2 years (at 6, 12, 18, and 24 months of age). In addition, blood samples for total and specific IgE analysis were drawn at 2 years of age. In figure 8, the time of data collection in present study is shown.

Figure 8 Time of data collection in the LISA study



2.2 Dietary assessment

2.2.1 Maternal diet during pregnancy

Maternal food intake during the last 4 weeks of pregnancy was assessed by using a 45-item, semiquantitative food-frequency questionnaire (FFQ) as part of the baseline questionnaire administered shortly after childbirth. For each food item, the mothers reported their average consumption frequency over the past 4 weeks according to five categories ranging from "< 2 times/month or never" to "≥ 4 times/week".

Based on this information, children were categorised into high and low intake groups according to maternal intake. Therefore, each food-frequency variable was dichotomised at a cut-off close to the second tertile. In this way, we contrasted the upper tertile (T3) with the combination of the lowest and the middle tertiles (T1-T2) for margarine, vegetable oil, deep-frying vegetable fat and fish intake. For butter intake, considerably more than 33% of all subjects were in the highest consumption category; therefore, this procedure was not feasible. The cut-off was set at the lowest tertile, and the combined middle and upper tertiles (T2-T3) were contrasted with the lowest tertile (T1). Thus, we had to use different reference categories for the analyses.

Depending on the intake distribution of each food item, high maternal intake referred to different consumption frequencies. The high intake category included subjects who consumed foods at least "2-3 times/month" (deep-frying vegetable fat), "1-2 times/week" (fish), "3-4 times/week" (butter, vegetable oils) or "≥ 4 times/week" (margarine).

2.2.2 Dietary intake in children at the age of 2 years

Information on dietary fat intake in children were based on parental report of how often they have used butter, margarine, vegetable oils and other fats for cooking, baking and preparing salad dressings at home in the past 6 months. The following six answers were possible: "(almost) daily", "several times a week", "about once a week", "two to three times a month", "once in a month or less", and "(almost) never". Furthermore, parents should specify the type of spread their child has consumed in the last 6 months: "Only butter", "only margarine", "more butter than margarine", "more margarine than butter", "the same amount of butter and margarine", or "neither of them". We aggregated similar answering categories and distinguished between "butter only", "margarine only", "butter and margarine" (in any case of combined spread) and "neither butter nor margarine".

Finally, total butter and margarine intake in children should be assessed. To avoid any loss of information, we combined frequencies of household consumption with the spread intake of the child. Butter and margarine intake in terms of household consumption frequencies were summarised and two main groups of frequent (more than once a week) and infrequent (once a week or less) household consumption established.

Three mutually exclusive exposure categories were defined:

1. Butter group: Children with frequent butter and infrequent margarine household consumption and only consuming butter as spread.
2. Margarine group: Children with frequent margarine and infrequent butter household consumption and only consuming margarine as spread.
3. Mixed group: The remaining group.

Children were categorised into two intake groups according to the reported household consumption of dietary fats. Therefore, each food-frequency variable was dichotomised at a cut-off close to the 80th percentile. In this way, we contrasted the upper quintile (Q5) with the combination of the lower quintiles (Q1-Q4) for margarine, safflower oil, rapeseed oil and deep-frying vegetable fat intake. For butter, olive oil and sunflower oil intake, considerably more than 20% of all subjects were in the highest consumption category. The cut-off was set at the 20th percentile, and the lowest quintile (Q1) was contrasted with the upper quintiles (Q2-Q5). Thus, we used different reference categories for the analyses.

Depending on the intake distribution of each food item, low intake (Q1) and high intake (Q5) each referred to different consumption frequencies. Q1 is corresponding to dietary fat intake not exceeding “once in a month or less” (butter) or “(almost) never” (sunflower oil, olive oil). With regard to high intake, Q5 includes children who consumed foods at least “(almost) daily” (margarine), “two to three times a month” (safflower oil, deep-frying vegetable fat) or “once in a month or less” (rapeseed oil).

2.3 Questionnaire data on socioeconomic status

Information on region and parental education was obtained from the birth-questionnaire, while household income was reported in the 2-year-questionnaire.

2.3.1 Parental education

Parental education was determined based on information about school education according to the German educational system, and was defined by the highest grade completed either by the

mother or by the father. Thus, children were assigned to the group of low (less than 10th grade), medium (10th grade), or high (more than 10th grade) level of parental education.

2.3.2 Equivalent income

Net household income per month was reported on an eleven-point scale ranging from less than DM 1000 to more than DM 6000 (1 DM is equal to 0.5113 €). Since this income measure does not account for the total number of household members and consequently does not reflect the actual amount that is available for each person, adjustment for family size and family composition was needed. The calculation of equivalent income according to the new OECD (Organisation for Economic Co-operation and development) guidelines (86) was performed by dividing the net household income by an equivalence factor, which gives a weight of 1.0 to the first adult, 0.5 to all other adult persons and children above 14, and 0.3 to all children up to 14. As income was measured categorically, we took the mid-point of each income class to calculate the income level. For the lowest income level (less than 511 €) we calculated two-thirds of this limit, and for the highest income level (more than 3068 €) four-thirds, as previously recommended (87). Finally, the new variable was collapsed into three groups each containing approximately an equal number of subjects. This resulted in the following groups of equivalent income: 160 €-913 € (low), 914 €-1339 € (medium), 1340 €-3146 € (high).

2.4 Blood sampling and IgE measurements

At 2 years of age, blood samples were drawn after written informed consent was obtained from the parents. About 10 ml of peripheral venous blood was taken from each subject and left to coagulate for up to 6 hours at room temperature before centrifuged at 3000 rpm for 10 minutes. The supernatant serum was aliquoted into 1.5 to 2.0 ml microtubes and stored at -20°C. All samples were sent to the same laboratory (University of Leipzig) for further analysis.

Total and specific serum IgE levels were assayed by using the CAP-RAST FEIA system (Pharmacia Diagnostics, Freiburg, Germany) according to the manufacturer's instructions. The limit of detection for specific IgE was 0.35 kU/l, and values ≥0.35 kU/l were considered positive. Total and specific IgE antibodies to food allergens (fx5), house dust allergens (hx2), cat dander (e1), mixed moulds (mx1), and seasonal allergens (rx1) were measured. The composition of the multi-allergen screening tests is shown in table 2.

Table 2 Composition of the multi-allergen screening tests used for specific IgE analyses

Mix	Single allergens
fx5	egg white (f1), cow milk (f2), codfish (f3), wheat flour (f4), peanut (f13), soybean (f14)
hx2	*Dermatophagoides pteronyssinus* (d1), *Dermatophagoides farinae* (d2), cockroach (i6), house dust/Hollister Steer Labs (h2)
mx1	*Penicillium notatum* (m1), *Cladosporium herbarum* (m2), *Aspergillus fumigatus* (m3), *Alternaria alternata* (m6)
rx1	timothy grass (g6), mugwort (w6), English plantain , ribwort (w9), wall pellitory (w21), birch pollen (t3)

A positive result in the food allergen test (fx5) was followed by measurement of the single allergens egg white (f1), cow milk (f2), and peanut (f13). Subjects with positive results in the house dust allergen test (hx2) were further tested for specific IgE antibodies against the house dust mites *Dermatophagoides pteronyssinus* (d1) and *Dermatophagoides farinae* (d2).

2.5 Outcome variables

Parental-completed questionnaires were used to gather information on symptoms and doctor-diagnosed allergic diseases. Allergic sensitisation was assessed by measuring specific serum IgE concentrations.

2.5.1 Allergic sensitisation

Allergic sensitisation against food allergens was defined as a specific serum IgE concentration ≥ 0.35 kU/l against food allergens (fx5). Allergic sensitisation against inhalant allergens was defined as a specific serum IgE concentration ≥ 0.35 kU/l against at least one of the following allergen mixes: house dust allergens (hx2), cat dander (e1), mixed moulds (mx1) or seasonal allergens (rx1).

2.5.2 Eczema

Eczema definitions are based on questionnaire-derived information obtained from the parents. Two different definitions for eczema were used. Symptomatic eczema was defined as itchy rash that was not restricted to the skin underneath the diaper, and that was either recurrent or persisted for more than 14 days. Doctor-diagnosed eczema was based on a positive answer to

the question: “Has a doctor diagnosed your child with allergic or atopic eczema in the past 6 months?”

Further differentiation was performed towards the time period in which the skin condition appeared for the first time. Lifetime prevalence of symptomatic and doctor-diagnosed eczema was assumed, if eczema has ever appeared within the first 2 years of life. Incidence of symptomatic and doctor-diagnosed eczema in the second year of life was defined as initial appearance between the age of one and two years.

2.6 Statistical analysis

Descriptive statistics were used to describe the study population and to detect any major differences in food intake between subjects with and subjects without allergic diseases. Simple logistic regression models were calculated to quantify these associations, and multiple logistic regression models were applied to estimate the influence of potential confounders. All computations were performed by using the statistical analysis package SAS for Windows version 8.2 and 9.1 (SAS Institute, Cary, NC, USA).

2.6.1 Descriptive analysis

In present study, explanatory and outcome variables are described by categorical data. Contingency tables are widely used in descriptive statistics to illustrate the association between two categorical variables (88). The statistical significance of the association can be tested with the chi-square test, which is based on a test statistic that measures the difference between the observed data and the values that would be expected under the null hypothesis of no association. The chi-square test statistic is

$$\chi^2 = \sum_i \sum_j \frac{(O_{ij} - E_{ij})^2}{E_{ij}},$$

where O_{ij} is the observed value in cell (i,j) and E_{ij} is the expected frequency in cell (i,j).

The null hypothesis is rejected whenever the p-value of the two-sided chi-square test is lower than 0.05 at the 5% level of significance, which is referred to as being statistically significant. The p-value is the probability of obtaining a result as extreme as that observed for the test statistic when the null hypothesis is actually true, so that the finding was the results of chance alone.

2.6.2 Logistic regression analysis

Logistic regression is a particular type of statistical models called generalised linear models (89). Linear regression models are usually used to describe an association between a dependent or outcome variable Y and one or more independent or explanatory variables X_1, X_2,…, X_m. The equation for linear regression models is given by

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_m X_m ,$$

where β_0, β_1, $\beta_{2,\ldots,}$ β_m are the unknown parameters.

In contrast to linear regression models, the dependent variable in logistic regression models is dichotomous, which means that it can only take two different qualitative outcomes, such as the presence or absence of a disease. Instead of modelling the probability of the diseases itself, the logistic regression model is characterised by using the logit of the probability P of a disease (90). The logit transformation is defined as the natural logarithm of the odds, which is the chance of a disease to occur:

$$\text{logit}(P) = \ln\left(\frac{P}{1-P}\right).$$

As the probability P can only take values greater than or equal to zero and less than or equal to one, the logit (P) may range from -∞ (=logit(0)) to +∞ (=logit(1)).

Modelling the logit (P) as a linear function of the independent variables, the logistic regression model takes the form

$$\text{logit}\ (P) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_{mX_m} .$$

This can be written equivalently as

$$P = \frac{\exp(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_m X_m)}{1 + \exp(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_m X_m)}.$$

The parameters β_0, β_1, $\beta_{2,\ldots,}$ β_m are estimated by the method of maximum likelihood yielding values for the unknown parameters, which maximise the probability of obtaining the observed set of data. They are called maximum likelihood estimates and are denoted as $\hat{\beta}_0$, $\hat{\beta}_1, \hat{\beta}_2, \ldots, \hat{\beta}_m$.

The results of logistic regression models are presented as odds ratio (OR). It is defined as the ratio of the odds for the outcome being present among those exposed to the risk factor $P(1)/[1\text{-}P(1)]$ to the odds for those unexposed $P(0)/[1\text{-}P(0)]$.

$$\mathrm{OR} = \frac{P(1)/\left[1-P(1)\right]}{P(0)/\left[1-P(0)\right]}$$

An OR of one indicates that the outcome under study is equally likely in the group of exposed and in the group of unexposed individuals. An OR above one implies that the exposure to the risk factor increases the odds of the outcome, while a value below one reduces the odds of the outcome. The relationship between the odds ratio and the parameter estimate is

$$\mathrm{OR} = \exp(\hat{\beta}j); \quad j=1,\ldots m.$$

The ORs are accompanied by their corresponding 95% confidence intervals (95% CI), specifying the range of values being 95% confident that it contains the true value.

3 SOCIOECONOMIC DETERMINANTS OF DIETARY FAT INTAKE

One objective of present work is to estimate the influence of socioeconomic factors on dietary fat intake in 2-year-old children. In the following, the impact of region, parental education and equivalent income on dietary fat intake will be described. The results presented in this section have been partly published in Sausenthaler S, Kompauer I, Mielck A, Borte M, Herbarth O, Schaaf B, Berg v A, Wichmann HE, Heinrich J for the LISA Study Group. *Impact of parental education and income inequality on children's food intake.* Public Health Nutrition 2007; 10:24-33.

3.1 Description of the study population

Socioeconomic characteristics of the study population are given in table 3. In brief, fifty percent and 29.8% of all children lived in the urban areas of Munich and Leipzig, respectively, while the rest lived in the more rural areas of Wesel and Bad Honnef. Compared to the total German population, parental education was high, as the majority of children were born to parents of whom either the mother or the father completed more than 10 grades (67.8%).

Table 3 Socioeconomic characteristics of the study population

	Frequency	
Variable	**n/N**	**%**
Study area		
Munich	1331/2664	*50.0*
Leipzig	794/2664	*29.8*
Wesel	271/2664	*10.2*
Bad Honnef	268/2664	*10.0*
Level of parental education		
Low (< 10^{th} grade)	120/2637	*4.5*
Medium (10^{th} grade)	729/2637	*27.7*
High (> 10^{th} grade)	1788/2637	*67.8*
Equivalent income (per month)		
Low (≤ 913 €)	763/2376	*32.1*
Medium (914 €-1339 €)	798/2376	*33.6*
High (≥ 1340 €)	815/2376	*34.3*

About one-third of the study population had an equivalent income of more than 1340 € and one-third less than 913 €.

3.2 Dietary fat intake in children at the age of 2 years

Table 4 shows the consumption frequencies of dietary fat in the study population. A large proportion of children consumed butter almost daily (35.2%) and margarine almost never (34.0%). Furthermore, the majority of the study population consumed olive oil (57.1%) and sunflower oil (62.7%) at least once a week. In contrast, safflower oil (75.0%), rapeseed oil (94.7%) and deep-frying vegetable fat (66.6%) were reported to be almost never consumed by most of the subjects.

Table 4 Consumption frequencies of dietary fat in the study population

Variable	N	(almost) never n *(%)*	1 x/ month or less n *(%)*	2-3 x/ month n *(%)*	about once a week n *(%)*	several times a week n *(%)*	(almost) daily n *(%)*
Butter	2636	339 *(12.9)*	159 *(6.0)*	212 *(8.0)*	377 *(14.3)*	620 *(23.5)*	929 *(35.3)*
Margarine	2616	890 *(34.0)*	164 *(6.3)*	179 *(6.8)*	279 *(10.7)*	551 *(21.1)*	553 *(21.1)*
Olive oil	2635	600 *(22.8)*	258 *(9.8)*	271 *(10.3)*	422 *(16.0)*	726 *(27.5)*	358 *(13.6)*
Sunflower oil	2613	520 *(19.9)*	181 *(6.9)*	273 *(10.5)*	499 *(19.1)*	915 *(35.0)*	225 *(8.6)*
Safflower oil	2566	1925 *(75.0)*	146 *(5.7)*	98 *(3.8)*	125 *(4.9)*	215 *(8.4)*	57 *(2.2)*
Rapeseed oil	2555	2419 *(94.7)*	59 *(2.3)*	22 *(0.8)*	17 *(0.7)*	33 *(1.3)*	5 *(0.2)*
Deep-frying vegetable fat	2576	1716 *(66.6)*	269 *(10.4)*	196 *(7.6)*	220 *(8.6)*	153 *(5.9)*	22 *(0.9)*

3.3 Dietary fat intake in relation to socioeconomic factors

3.3.1 Region and dietary fat intake

Dietary fat intake significantly varied between the different study areas as shown in table 5. In particular, low butter and high margarine intake were seen more often in Wesel/Bad Honnef (32.5% and 37.4%) than in Leipzig (19.5% and 31.5%) and Munich (13.0% and 8.2%). Furthermore, low olive oil intake was more frequently reported in Leipzig (35.1%) and Wesel/Bad Honnef (36.3%) than in Munich (10.0%). Low sunflower oil intake showed a slight decrease from the urban areas of Munich (20.9%) and Leipzig (21.3%) to the more rural areas of Wesel/Bad Honnef (15.4%), while for safflower oil and deep-frying vegetable fat intake an East-West divide with higher intakes in Munich (24.7% and 23.5%) and

Wesel/Bad Honnef (22.9% and 27.0%) compared to Leipzig (7.6% and 19.3%) could be observed. Rapeseed oil intake, in contrast, did not differ substantially between the study areas.

Table 5 Dietary fat intake in children at 2 years of age according to region

	Munich (N=1331)		Leipzig (N=794)		Wesel/ Bad Honnef (N=539)		
Variable	**n/N**	**%**	**n/N**	**%**	**n/N**	**%**	**p-value[1]**
Low intake (Q1)[2]							
Butter	172/1319	*13.0*	153/785	*19.5*	173/532	*32.5*	<0.001
Olive oil	132/1321	*10.0*	274/780	*35.1*	194/534	36.3	<0.001
Sunflower oil	273/1308	*20.9*	166/780	*21.3*	81/525	*15.4*	0.016
High intake (Q5)[3]							
Margarine	107/1299	*8.2*	246/782	*31.5*	200/535	*37.4*	<0.001
Safflower oil	317/1284	*24.7*	57/754	*7.6*	121/528	*22.9*	<0.001
Rapeseed oil	66/1285	*5.1*	47/747	*6.3*	23/523	*4.4*	0.306
Deep-frying vegetable fat	304/1295	*23.5*	146/758	*19.3*	141/523	*27.0*	0.005

[1] Chi-Square test; [2] lowest quintile of intake; [3] highest quintile of intake

3.3.2 Parental education and dietary fat intake

Table 6 shows the proportion of children with low and high dietary fat intake according to level of parental education. Low butter, high margarine and high deep-frying vegetable fat intake significantly decreased with increasing parental education. Furthermore, low olive oil intake was reported more than twice as often in the group of low and medium parental education than in the group of high parental education. Children with low or high parental education more frequently showed a high intake of safflower oil than did children with medium parental education. No statistically significant differences were observed for sunflower oil and rapeseed oil intake.

Table 6 Dietary fat intake in children at 2 years of age according to parental education

	Level of parental education						
	Low (N=120)		Medium (N=729)		High (N=1788)		
Variable	**n/N**	**%**	**n/N**	**%**	**n/N**	**%**	**p-value[1]**
Low intake (Q1)[2]							
Butter	33/117	*28.2*	166/721	*23.0*	293/1771	*16.5*	<0.001
Olive oil	44/118	*37.3*	281/715	*39.3*	263/1775	14.8	<0.001
Sunflower oil	18/115	*15.7*	136/712	*19.1*	359/1759	*20.4*	0.392
High intake (Q5)[3]							
Margarine	43/118	*36.4*	238/716	*33.2*	258/1756	*14.7*	<0.001
Safflower oil	23/112	*20.5*	95/700	*13.6*	374/1729	*21.6*	<0.001
Rapeseed oil	8/111	*7.2*	29/696	*4.2*	96/1722	*5.6*	0.240
Deep-frying vegetable fat	32/114	*28.1*	190/700	*27.1*	359/1735	*20.7*	0.001

[1] Chi-Square test; [2] lowest quintile of intake; [3] highest quintile of intake

3.3.3 Equivalent income and dietary fat intake

With increasing equivalent income, subjects less likely reported a low intake of butter and olive oil, and a high intake of margarine (table 7). High intake of safflower oil was more prevalent in medium- and high-income families than in low-income families. Intake of sunflower oil, rapeseed oil and deep-frying vegetable fat seemed not to be determined by equivalent income.

Table 7 Dietary fat intake in children at 2 years of age according to equivalent income

	Equivalent income						
	Low (N=763)		Medium (N=798)		High (N=815)		
Variable	**n/N**	**%**	**n/N**	**%**	**n/N**	**%**	**p-value[1]**
Low intake (Q1)[2]							
Butter	178/752	*23.7*	160/794	*20.2*	112/804	*13.9*	<0.001
Olive oil	266/752	*35.4*	181/790	*22.9*	68/808	8.4	<0.001
Sunflower oil	136/746	*18.2*	143/782	*18.3*	176/802	*21.9*	0.103
High intake (Q5)[3]							
Margarine	239/749	*31.9*	172/788	*21.8*	75/798	*9.4*	<0.001
Safflower oil	102/726	*14.0*	161/781	*20.6*	167/785	*21.3*	<0.001
Rapeseed oil	33/725	*4.6*	42/775	*5.4*	46/781	*5.9*	0.504
Deep-frying vegetable fat	167/729	*22.9*	187/778	*24.0*	159/789	*20.2*	0.165

[1] Chi-Square test; [2] lowest quintile of intake; [3] highest quintile of intake

3.4 Association between dietary fat intake and socioeconomic factors

To investigate the association of dietary fat intake with socioeconomic determinants, three different models have been computed. First, the crude association of dietary fat intake with parental education and equivalent income was examined. To consider potential confounding by regional differences in exposure and outcome, the model was adjusted for study area. Finally, a model that includes study area, parental education and equivalent income was applied. Associations were considered, if at least in one group the effect estimate was statistically significant and, if the effect estimates showed the same direction across all categories of socioeconomic status. An independent contribution of this socioeconomic indicator to dietary fat intake was assumed, if the fully adjusted effect estimate was statistically significant.

Table 8 shows the logistic regression results for the association between dietary fat intake and parental education. Considering unadjusted effects, low intake of butter and high intake of margarine steadily increased with decreasing levels of parental education. In children with medium or low parental education, we more likely observed low olive oil and high deep-frying vegetable fat intake compared to children with high parental education, although the effect was borderline significant for deep-frying vegetable fat. In turn, high safflower intake

showed a reduced odds ratio in the group of medium parental education only. Sunflower oil and rapeseed oil intake seemed not to be determined by parental education.

When including study area in the multiple logistic regression models, the relative change in the odds ratios gives information about the influence of regional differences on the association between parental education and dietary fat intake. Study area showed to have a weak influence (10-20% relative change) on butter consumption, and a very strong one (> 20% relative change) on the intake of olive oil and margarine. After adjustment, effect estimates diminished but remained statistically significant except for safflower oil intake.

Table 8 Logistic regression results describing the association between parental education and dietary fat intake in 2-year old children

		Level of parental education		
		High (N=1788)[1]	Medium (N=729)	Low (N=120)
Variable		**OR**	**OR (95% CI)**	**OR (95% CI)**
Low intake (Q1)[2]				
Butter	OR[3]	1.00	1.51 (1.22-1.87)[7]	1.98 (1.30-3.02)[7]
	aOR[4]		1.33 (1.06-1.66)[7]	1.66 (1.07-2.56)[7]
	aOR[5]		1.16 (0.90-1.48)	1.53 (0.95-2.46)
Olive oil	OR[3]	1.00	3.72 (3.05-4.54)[7]	3.42 (2.30-5.08)[7]
	aOR[4]		2.81 (2.28-3.46)[7]	3.10 (2.04-4.71)[7]
	aOR[5]		2.26 (1.79-2.86)[7]	2.29 (1.44-3.64)[7]
Sunflower oil	OR[3]	1.00	0.92 (0.74-1.15)	0.72 (0.43-1.21)
	aOR[4]		0.92 (0.73-1.16)	0.77 (0.46-1.29)
	aOR[5]		0.97 (0.75-1.25)	0.87 (0.50-1.53)
High intake (Q5)[6]				
Margarine	OR[3]	1.00	2.89 (2.36-3.55)[7]	3.33 (2.24-4.95)[7]
	aOR[4]		2.12 (1.71-2.64)[7]	2.86 (1.87-4.38)[7]
	aOR[5]		1.78 (1.39-2.26)[7]	2.58 (1.61-4.11)[7]
Safflower oil	OR[3]	1.00	0.67 (0.45-0.73)[7]	0.94 (0.58-1.50)
	aOR[4]		0.74 (0.57-0.95)[7]	0.90 (0.56-1.45)
	aOR[5]		0.72 (0.59-1.04)	0.72 (0.41-1.27)
Rapeseed oil	OR[3]	1.00	0.74 (0.48-1.13)	1.32 (0.62-2.78)
	aOR[4]		0.69 (0.45-1.08)	1.36 (0.64-2.90)
	aOR[5]		0.72 (0.45-1.17)	0.83 (0.29-2.36)
Deep-frying vegetable fat	OR[3]	1.00	1.43 (1.17-1.75)[7]	1.50 (0.98-2.29)
	aOR[4]		1.55 (1.26-1.92)[7]	1.44 (0.94-2.20)
	aOR[5]		1.50 (1.19-1.91)[7]	1.57 (0.98-2.50)

[1] Reference category; [2] lowest quintile of intake; [3] crude odds ratios; [4] Odds ratios adjusted for study area;
[5] Odds ratios adjusted for study area and equivalent income; [6] highest quintile of intake; [7] $p<0.05$

When additionally adjusting for equivalent income, odds ratios for olive oil and margarine intake somewhat decreased, and attenuated to non-significance for butter intake.

Table 9 shows the logistic regression results for the association between dietary fat intake and equivalent income. The crude odds ratios suggest that the likelihood of low butter and olive oil intake, and a high margarine intake increases with decreasing equivalent income. Children with low equivalent income also less likely reported a high intake of rapeseed oil. No statistically significant differences could be detected for sunflower oil, safflower oil and deep-frying vegetable fat.

Table 9 Logistic regression results describing the association between equivalent income and dietary fat intake in 2-year old children

		Equivalent income		
		High (N=815)[1]	Medium (N=798)	Low (N=763)
Variable		**OR**	**OR (95% CI)**	**OR (95% CI)**
Low intake (Q1)[2]				
Butter	OR[3]	1.00	1.56 (1.20-2.03)[7]	1.92 (1.48-2.49)[7]
	aOR[4]		1.34 (1.02-1.75)[7]	1.57 (1.18-2.08)[7]
	aOR[5]		1.26 (0.96-1.67)	1.39 (1.02-1.88)[7]
Olive oil	OR[3]	1.00	3.23 (2.40-4.36)[7]	5.96 (4.46-7.96)[7]
	aOR[4]		2.52 (1.85-3.43)[7]	3.73 (2.75-5.07)[7]
	aOR[5]		2.10 (1.53-2.88)[7]	2.65 (1.91-3.67)[7]
Sunflower oil	OR[3]	1.00	0.80 (0.62-1.02)	0.79 (0.62-1.02)
	aOR[4]		0.80 (0.63-1.03)	0.78 (0.59-1.02)
	aOR[5]		0.80 (0.62-1.03)	0.78 (0.59-1.05)
High intake (Q5)[6]				
Margarine	OR[3]	1.00	2.69 (2.01-3.60)[7]	4.52 (3.40-6.00)[7]
	aOR[4]		2.01 (1.48-2.73)[7]	2.68 (1.98-3.63)[7]
	aOR[5]		1.71 (1.25-2.34)[7]	1.93 (1.39-2.67)[7]
Safflower oil	OR[3]	1.00	0.92 (0.60-1.41)	0.76 (0.48-1.21)
	aOR[4]		0.87 (0.56-1.35)	0.66 (0.40-1.08)
	aOR[5]		0.93 (0.59-1.45)	0.71 (0.42-1.21)
Rapeseed oil	OR[3]	1.00	0.96 (0.75-1.23)	0.61 (0.46-0.79)[7]
	aOR[4]		1.13 (0.88-1.45)	0.94 (0.70-1.25)
	aOR[5]		1.16 (0.90-1.50)	1.04 (0.76-1.42)
Deep-frying vegetable fat	OR[3]	1.00	1.25 (0.99-1.59)	1.18 (0.92-1.50)
	aOR[4]		1.29 (1.01-1.65)[7]	1.31 (1.00-1.70)[7]
	aOR[5]		1.19 (0.93-1.53)	1.09 (0.82-1.45)

[1] Reference category; [2] lowest quintile of intake; [3] crude odds ratios; [4] Odds ratios adjusted for study area; [5] Odds ratios adjusted for study area and parental education; [6] highest quintile of intake; [7] $p<0.05$

The adjusted odds ratios indicate a strong influence of study area on the association between equivalent income and the intake of butter, olive oil and margarine. In the fully adjusted model, associations remained significant for butter, olive oil and margarine intake.

Associations that remained statistically significant after mutually adjustment, were considered independent. Thus, parental education seems to have an independent influence on the intake of olive oil, margarine and deep-frying vegetable fat, while equivalent income seems to influence intake of butter, olive oil and margarine independently.

4 DIETARY FAT INTAKE AND ALLERGIC DISEASES

Another objective of present work was to analyse the association between dietary fat intake and allergic diseases in children. For this purpose, prenatal dietary exposure, which is represented by maternal diet during pregnancy, and dietary exposure at 2 years of age was analysed for associations with allergic outcomes at 2 years of age. Because allergic rhinoconjunctivitis and asthma predominantly develop later in life (23), present study focused on childhood eczema and allergic sensitisation , which are most often the first manifestation of allergic diseases.

4.1 Maternal dietary fat intake during pregnancy in relation to eczema and allergic sensitisation in the offspring at 2 years of age

The results presented in this section have already been published as part of Sausenthaler S, Koletzko S, Schaaf B et al. *Maternal food intake during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 years of age*. Am J Clin Nutr 2007; 85:530-7.

4.1.1 Description of the study population

The frequency of basic characteristics and possible confounding factors in the study population is shown in table 10. In brief, fifty percent of the study population lived in Munich and slightly more than half of the children were boys (52%). Mean maternal age at delivery was 31 years (standard deviation (SD) = 4 years). About 10% of all mothers smoked during the second and/or the third trimester of pregnancy. Parental education was very high, as in 54.6% of all children either the mother or the father has graduated from university. More than half of the children were exclusively breastfed for at least 4 months (57.6%) and the majority of children had a positive parental history of atopic diseases with at least one parent having asthma, hay fever or eczema (52.7%). The majority of children was first-born (56.3%) and less than 10% had two older siblings or more. The mean birth weight was 3472 g (SD=442 g). Children were somewhat less likely born in spring (21.7%) than in the other seasons.

Table 10 Basic characteristics of the study population[1] at the age of 2 years

Variable	Frequency n/N	Frequency %
Study area		
Munich	1320/2641	*50.0*
Leipzig	785/2641	*29.7*
Wesel	269/2641	*10.2*
Bad Honnef	267/2641	*10.1*
Gender		
Male	1360/2641	*52.0*
Female	1281/2641	*48.0*
Maternal age at delivery		
≤ 31 years	1371/2639	*52.0*
> 31 years	1268/2639	*48.0*
Maternal smoking during pregnancy[2]		
No	2272/2528	*89.9*
Yes	256/2528	*10.1*
Level of parental education		
Very high	1430/2617	*54.6*
High	536/2617	*20.5*
Medium	522/2617	*20.0*
Low	129/2617	*4.9*
Exclusively breastfed for at least 4 months		
Yes	1506/2616	*57.6*
No	1110/2616	*42.4*
Parental history of atopic diseases[3]		
None	1248/2636	*47.3*
One parent atopic	1030/2636	*39.1*
Both parents atopic	358/2636	*13.6*
Number of older siblings		
0	1486/2641	*56.3*
1	895/2641	*33.9*
≥ 2	260/2641	*9.8*
Birth weight		
2500-3250 g	871/2641	*33.0*
3251-3650 g	897/2641	*34.0*
3651-5190 g	873/2641	*33.0*
Season of birth		
Spring (Mar-May)	572/2641	*21.7*
Summer (June-Aug)	722/2641	*27.3*
Fall (Sept-Nov)	696/2641	*26.4*
Winter (Dec-Feb)	651/2641	*24.6*

[1] Subjects without dietary information were excluded; [2] during 2nd/3rd trimester; [3] asthma, hay fever or eczema

4.1.2 Maternal dietary fat intake during pregnancy

Maternal consumption frequencies of foods rich in dietary fat are shown in table 11. During the last 4 weeks of pregnancy, more than half of mothers frequently (more than 4 times a week) consumed butter and about one-third frequently consumed margarine. The majority of mothers consumed vegetable oils at least once a week (61.3%). In turn, the intake of deep-frying vegetable fat was low with 65% reporting a frequency of less than two times a month. About one-third of the study population indicated to consume fish at least once per week.

Table 11 Consumption frequencies of maternal dietary fat intake during the last 4 weeks of pregnancy

		Consumption frequency									
		< 2 times a month		2-3 times a month		1-2 times a week		3-4 times a week		> 4 times a week	
Variable	**N**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Butter	2591	396	*15.3*	221	*8.5*	297	*11.5*	238	*9.2*	1439	*55.5*
Margarine	2537	1015	*40.0*	206	*8.1*	251	*9.9*	218	*8.6*	847	*33.4*
Vegetable oils	2612	553	*21.2*	458	*17.5*	776	*29.7*	490	*18.8*	335	*12.8*
Deep-frying vegetable fat	2582	1678	*65.0*	380	*14.7*	339	*13.1*	127	*4.9*	58	*2.3*
Fish	2619	659	*25.2*	1154	*44.1*	785	*30.0*	17	*0.6*	4	*0.1*

4.1.3 Prevalence of eczema and allergic sensitisation in the offspring at 2 years of age

Table 12 shows the outcome prevalence in the study population. At 2 years of age, 17.7% of all children have ever had doctor-diagnosed eczema, 9.3% were sensitised against food allergens, and 4.8% were sensitised against inhalant allergens. Of those sensitised against food allergens, milk (5.1%) and egg sensitisation (5.4%) was most common. Sensitisation to inhalant allergens was mainly ascribed to house dust allergens (2.8%).

Table 12 Prevalence of eczema and allergic sensitisation in children at the age of 2 years

Variable	Frequency n/N	Frequency %
Doctor-diagnosed eczema[1]	446/2518	*17.7*
Allergic sensitisation[2] against		
Any (food or inhalant) allergen	264/2139	*12.3*
Food allergens	200/2146	*9.3*
Cow's milk	110/2144	*5.1*
Egg	116/2144	*5.4*
Peanut	37/2143	*1.7*
Inhalant allergens	103/2138	*4.8*
House dust	59/2143	*2.8*
Cat dander	26/2138	*1.2*
Mixed moulds	9/2139	*0.4*
Seasonal allergens	32/2140	*1.5*

[1] Lifetime prevalence; [2] IgE ≥ 0.35kU/l

The prevalence of eczema and allergic sensitisation according to the categories of maternal dietary fat intake is shown in table 13. Children of mothers with a high intake of margarine and vegetable oils during pregnancy had a statistically significant higher lifetime prevalence of doctor-diagnosed eczema at 2 years of age than did children whose mothers had a low intake of these foods. Allergic sensitisation against inhalant allergens was more prevalent in children whose mothers had a high intake of deep-frying vegetable fat during pregnancy than did those whose mothers had a low intake. No differences in the prevalence of allergic sensitisation against food allergens were observed between low and high dietary fat intake of the mother.

Table 13 Prevalence of eczema and allergic sensitisation at the age of 2 years according to maternal dietary fat intake during pregnancy

Variable	Intake category	Eczema			Allergic sensitisation against								
					Any allergens			Food allergens			Inhalant allergens		
		n/N	%	p^1	n/N	%	p^1	n/N	%	P^1	n/N	%	p^1
Butter	low	162/857	*18.9*		96/753	*12.7*		73/756	*9.7*		40/752	*5.3*	
	high	274/1616	*17.0*	0.227	163/1350	*12.1*	0.652	122/1354	*9.0*	0.623	63/1350	*4.7*	0.507
Margarine	low	267/1626	*16.4*		171/1378	*12.4*		131/1382	*9.5*		66/1377	*4.8*	
	high	163/801	*20.3*	0.017	82/691	*11.9*	0.722	62/694	*8.9*	0.687	32/691	*4.6*	0.870
Vegetable oils	low	281/1703	*16.5*		182/1465	*12.4*		138/1470	*9.4*		71/1464	*4.9*	
	high	160/789	*20.3*	0.022	80/653	*12.3*	0.912	60/655	*9.2*	0.868	32/653	*4.8*	0.960
Deep-frying vegetable	low	281/1605	*17.5*		161/1353	*11.9*		127/1357	*9.4*		56/1352	*4.1*	
fat	high	157/860	*18.3*	0.643	98/739	*13.3*	0.366	69/742	*9.3*	0.964	45/739	*6.1*	0.047
Fish	low	322/1727	*18.6*		177/1458	*12.1*		133/1464	*9.1*		70/1457	*4.8*	
	high	122/771	*15.8*	0.088	85/663	*12.8*	0.659	66/664	*9.9*	0.530	32/663	*4.8*	0.982

[1] Chi-Square test

4.1.4 Association between maternal dietary fat intake during pregnancy and allergic diseases in the offspring

Results of the logistic regression models are presented in table 14. Potential confounders included in the model were study area, gender, maternal age at delivery, smoking during second and/or third trimester of pregnancy, parental education, exclusive breastfeeding for at least 4 months, parental history of atopy, season of birth and all dietary variables included in the FFQ. The effect estimates did not differ substantially between the crude and adjusted models indicating that potential confounders included in the model do not have a strong influence. The adjusted effects suggest that a high maternal intake of margarine and vegetable oils during pregnancy are both positively associated with doctor-diagnosis of eczema in the offspring. In turn, high maternal fish intake decreased the risk for eczema. The risk for allergic sensitisation against inhalant allergens was positively related to a high maternal intake of deep-frying vegetable fat during pregnancy.

Table 14 Logistic regression results describing the association between high maternal dietary fat intake during pregnancy and eczema and allergic sensitisation in their offspring at the age of 2 years

		Eczema	Allergic sensitisation[1] against		
			Any allergen	Food allergens	Inhalant allergens
Variable		**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Butter	OR[2]	0.88 (0.71-1.09)	0.94 (0.72-1.23)	0.93 (0.68-1.26)	0.87 (0.58-1.31)
	aOR[3]	1.08 (0.79-1.46)	0.97 (0.66-1.42)	0.93 (0.60-1.43)	0.86 (0.48-1.53)
Margarine	OR[2]	1.30 (1.05-1.62)[4]	0.95 (0.72-1.26)	0.94 (0.68-1.29)	0.97 (0.63-1.49)
	aOR[3]	1.49 (1.08-2.04)[4]	0.85 (0.56-1.27)	0.80 (0.50-1.27)	0.93 (0.50-1.73)
Vegetable oils	OR[2]	1.29 (1.04-1.60)[4]	0.98 (0.74-1.30)	0.97 (0.71-1.34)	1.01 (0.66-1.55)
	aOR[3]	1.48 (1.14-1.91)[4]	0.88 (0.63-1.25)	0.91 (0.61-1.34)	0.89 (0.53-1.51)
Deep-frying	OR[2]	1.05 (0.85-1.31)	1.13 (0.87-1.48)	0.99 (0.73-1.35)	1.50 (1.00-2.25)[4]
vegetable fat	aOR[3]	1.10 (0.87-1.41)	1.25 (0.92-1.70)	1.12 (0.79-1.58)	1.61 (1.02-2.54)[4]
Fish	OR[2]	0.82 (0.65-1.03)	1.06 (0.81-1.40)	1.11 (0.81-1.51)	1.01 (0.66-1.54)
	aOR[3]	0.75 (0.57-0.98)[4]	1.02 (0.73-1.43)	1.01 (0.69-1.48)	0.94 (0.56-1.57)

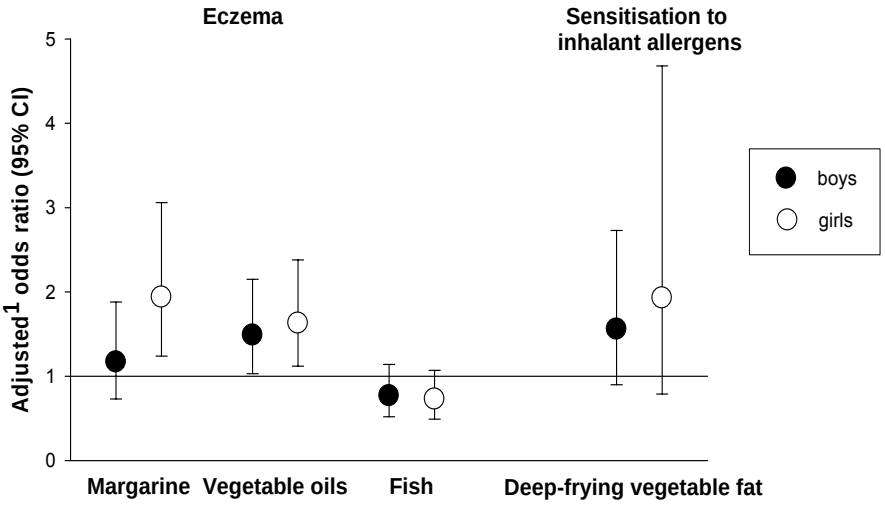
[1] IgE $\geq$ 0.35 kU/l; [2] Crude odds ratios; [3] Odds Ratios adjusted for study area, gender, maternal age at delivery, smoking during 2nd/3rd trimester of pregnancy, parental education, exclusive breastfeeding for at least 4 months, parental history of atopy, season of birth, dietary variables; [4] $p<0.05$

4.1.5 Sensitivity analyses

All associations, which turned out to be statistically significant in the adjusted logistic regression models, were then analysed stratified for gender, parental history of atopy and time of exclusive breastfeeding to look for potential effect modification.

Figure 9 shows adjusted logistic regression results for maternal dietary fat intake and eczema and allergic sensitisation to inhalant allergens stratified for gender. In girls, the risk for eczema was higher compared to boys in case their mothers had a high intake of margarine or vegetable oils during pregnancy. For fish intake, no gender-specific differences were observed. The positive association between high maternal intake of deep-frying vegetable fat and sensitisation to inhalant allergens was also somewhat more pronounced in girls than in boys, even though both effect estimates attenuated to non-significance due to small numbers. Overall, none of the associations showed opposite directions between boys and girls.

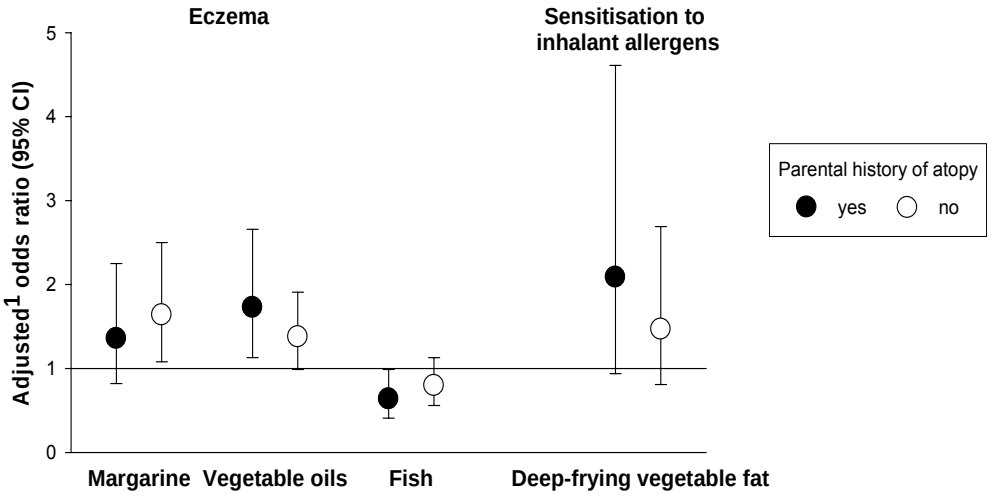
Figure 9 Adjusted logistic regression results describing the association between maternal dietary fat intake during pregnancy and eczema and allergic sensitisation to inhalant allergens in the offspring at 2 years of age stratified for gender



[1] Odds Ratios adjusted for study area, maternal age at delivery, smoking during 2nd/3rd trimester of pregnancy, parental education, exclusive breastfeeding for at least 4 months, season of birth, parental history of atopy, dietary variables

In addition, we looked for effect modification by parental history of atopic diseases. Results of the multiple logistic regression analysed stratified for parental atopy are given in figure 10. No systematic differences in the effects of maternal dietary fat intake were found between children with and children without parental atopy.

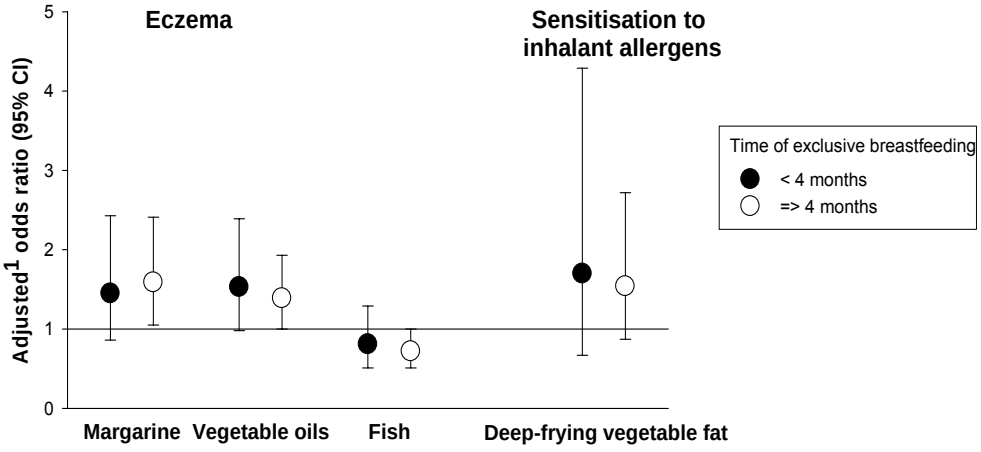
Figure 10 Adjusted logistic regression results describing the association between maternal dietary fat intake during pregnancy and eczema and allergic sensitisation to inhalant allergens in the offspring at 2 years of age stratified for parental history of atopy



[1] Odds Ratios adjusted for study area, gender, maternal age at delivery, smoking during 2nd/3rd trimester of pregnancy, parental education, exclusive breastfeeding for at least 4 months, season of birth, dietary variables

Stratification for time of exclusive breastfeeding revealed that no consistent differences in the effects of high maternal dietary fat intake exist between children who were exclusively breastfed for less than 4 months and those who were breastfed for at least 4 months. The results are shown in figure 11.

Figure 11 Adjusted logistic regression results describing the association between maternal dietary fat intake during pregnancy and eczema and allergic sensitisation to inhalant allergens in the offspring at 2 years of age stratified for time of exclusive breastfeeding



[1] Odds Ratios adjusted for study area, gender, maternal age at delivery, smoking during 2nd/3rd trimester of pregnancy, parental education, parental history of atopy, season of birth, dietary variables

4.2 Butter and margarine intake in childhood in relation to eczema and allergic sensitisation at 2 years of age

The association of butter and margarine intake with eczema and allergic sensitisation was analysed within a study population of 2582 children at the age of 2 years. The results presented here have already been published in Sausenthaler S, Kompauer I, Borte M et al. *Margarine and butter consumption, eczema and allergic sensitization. The LISA birth cohort study*. Pediatr Allergy Immunol 2006; 17: 85-93.

4.2.1 Description of the study population

Basic characteristics and potential confounding factors of the study population according to the exposure categories are shown in table 18. Overall, almost a third of the study population (30.3%) predominantly consumed butter ("butter group"), while only 7.3% of the children predominantly consumed margarine ("margarine group"). The majority of children (62.4%) either frequently consumed both butter and margarine or none of them ("mixed group").

Children living in Munich or Bad Honnef more likely consumed butter than margarine, while children in Wesel favoured margarine over butter. In Leipzig, no difference in the frequency of butter and margarine consumption was seen. Compared to the margarine group, a higher proportion of children in the butter group were born to mothers who were older than 31 years at delivery and were exclusively breastfed for at least 4 months. Children with butter consumption were less likely born to mothers who smoked during pregnancy but had more likely a positive parental history of atopic diseases or eczema. Butter intake also clearly increased with increasing parental education, whereas margarine intake decreased. Children with predominant margarine intake less likely reported to eat fresh fruit and salad and raw vegetables at least daily, and more likely kept cats or dogs in their home than children with predominant butter intake did. However, we could not observe any differences in gender, number of older siblings, birth weight and body mass index between children consuming butter and those consuming margarine.

Table 18 Basic characteristics of the study population[1] at the age of 2 years according to the exposure categories

		Mixed group (N=1612)		Butter group (N=782)		Margarine group (N=188)	
	N	n	%	n	%	n	%
Study area							
Munich	1285	661	*51.4*	607	*47.2*	17	*1.3*
Leipzig	772	586	*75.9*	94	*12.2*	92	*11.9*
Wesel	263	192	*73.0*	22	*8.4*	49	*18.6*
Bad Honnef	262	173	*66.0*	59	*22.5*	30	*11.5*
Gender							
Male	1327	827	*62.3*	410	*30.9*	90	*6.8*
Female	1255	785	*62.6*	372	*29.6*	98	*7.8*
Maternal age at delivery							
≤ 31 years	1352	898	*66.4*	324	*24.0*	130	*9.6*
> 31 years	1228	712	*58.0*	458	*37.3*	58	*4.7*
Maternal smoking during pregnancy[2]							
No	2223	1370	*61.6*	696	*31.3*	157	*7.1*
Yes	246	165	*67.1*	57	*23.2*	24	*9.7*
Level of parental education							
Very high	1392	819	*58.8*	525	*37.7*	48	*3.5*
High	526	341	*64.8*	134	*25.5*	51	*9.7*
Medium	509	350	*68.8*	95	*18.6*	64	*12.6*
Low	126	84	*66.6*	21	*16.7*	21	*16.7*
Exclusively breastfed for at least 4 months							
No	1086	699	*64.4*	276	*25.4*	111	*10.2*
Yes	1468	889	*60.6*	503	*34.3*	76	*5.2*
Parental history of atopic diseases[3]							
None	1216	786	*64.6*	325	*26.7*	105	*8.6*
One parent atopic	1015	621	*61.2*	333	*32.8*	61	*6.0*
Both parents atopic	346	204	*59.0*	121	*35.0*	21	*6.1*
Parental history of eczema							
No	2046	1283	*62.7*	608	*29.7*	155	*7.6*
Yes	351	212	*60.4*	124	*35.3*	15	*4.3*
Number of older siblings							
0	1456	898	*61.7*	452	*31.0*	106	*7.3*
1	873	547	*62.7*	257	*29.4*	69	*7.9*
≥ 2	253	167	*66.0*	73	*28.9*	13	*5.1*
Birth weight							
2500-3249 g	816	505	*61.9*	256	*31.4*	55	*6.7*
3250-3649 g	876	567	*64.7*	258	*29.5*	51	*5.8*
3650-5200 g	890	540	*60.7*	268	*30.1*	82	*9.2*

Table 18 *continued*

		Mixed group (N=1612)		Butter group (N=782)		Margarine group (N=188)	
	N	**n**	**%**	**n**	**%**	**n**	**%**
Body mass index[4]							
Normal weight	2414	1513	*62.7*	728	*30.1*	173	*7.2*
Overweight	155	91	*58.7*	50	*32.3*	14	*9.0*
Obesity	13	8	*61.5*	4	*30.8*	1	*7.7*
Fresh fruit intake							
≥ daily	2034	1260	*61.9*	638	*31.4*	136	*6.7*
< daily	512	327	*63.9*	137	*26.8*	48	*9.3*
Salad and raw vegetable intake							
≥ daily	753	473	*62.8*	249	*33.1*	31	*4.1*
< daily	1553	966	*62.2*	455	*29.3*	132	*8.5*
Keeping a cat							
No	2193	1365	*62.2*	694	*31.7*	134	*6.1*
Yes	262	167	*63.7*	57	*21.8*	38	*14.5*
Keeping a dog							
No	2251	1399	*62.1*	704	*31.3*	148	*6.6*
Yes	204	133	*65.2*	47	*23.0*	24	*11.8*

[1] Subjects without dietary information were excluded; [2]During 2nd/3rd trimester; [3] asthma, hay fever or eczema; [4] body mass index according to Cole et al (91) for boys (girls) at the age of 2 years: normal weight: <18.41 (18.02) kg/m^2; overweight: 18.41-20.07 (18.02-19.80) kg/m^2; obesity ≥ 20.08 (19.81) kg/m^2

4.2.2 Prevalence of allergic sensitisation and eczema at 2 years of age

The incidence rate of eczema in the second year of life was 9.7% for symptomatic eczema and 7.5% for doctor-diagnosed eczema in the study population. Lifetime prevalence was 20.9% for symptomatic eczema and 17.7% for doctor-diagnosed eczema. Sensitisation against food allergens was more prevalent (9.3%) than sensitisation against inhalant allergens (4.8%). Overall, 12.3% of the children were sensitised against any (food or inhalant) allergen.

Table 19 shows the prevalence rates in the different exposure categories. The incidence of symptomatic eczema in the second year was higher in the butter group (11.4%) than in the mixed (8.9%) and the margarine group (9.4%). The highest prevalence for all other outcome variables was seen in children with predominant margarine consumption. At the same time there was a large discrepancy between the frequency of reported symptoms and doctor-diagnosed eczema in the butter group, but not in the margarine group.

Table 19 Prevalence rates for eczema and allergic sensitisation in the LISA-study population at the age of 2 years according to the exposure categories

Variable	Mixed group (N=1612)		Butter group (N=782)		Margarine group (N=188)	
	n/N	%	n/N	%	n/N	%
Eczema symptoms						
Incidence in the second year of life	115/1297	*8.9*	73/639	*11.4*	13/139	*9.4*
Lifetime prevalence	297/1479	*20.1*	151/717	*21.1*	47/173	*27.2*
Doctor-diagnosed eczema						
Incidence in the second year of life	99/1375	*7.2*	53/678	*7.8*	13/137	*9.5*
Lifetime prevalence	262/1538	*17.0*	129/754	*17.1*	45/169	*26.6*
Allergic sensitisation[1] against						
Food or inhalant allergens	158/1315	*12.0*	73/631	*11.6*	26/151	*17.2*
Food allergens	120/1322	*9.1*	57/631	*9.0*	18/151	*11.9*
Inhalant allergens	59/1314	*4.5*	27/631	*4.3*	14/151	*9.3*

[1] IgE ≥ 0.35 kU/l

4.2.3 Association between butter and margarine intake and allergic diseases in 2-year-old children

Results from the logistic regression analyses are presented in table 20. Crude (OR) and adjusted odds ratios (aOR) with 95% confidence intervals (95% CI) were computed for the butter and the margarine group compared to the mixed group. The adjusted model included study area (Munich/Leipzig/Wesel/Bad Honnef), gender, maternal age at delivery (≤31 years/>31 years), maternal smoking during second or third trimester of pregnancy, level of parental education (very high/high/medium/low), exclusive breast-feeding for at least 4 months, parental history of atopic diseases (asthma, hay fever or eczema) (none/one parent atopic/both parents atopic), fresh fruit intake (≥daily/<daily), salad and raw vegetable intake (≥daily/<daily), keeping a dog (yes/no), and keeping a cat (yes/no).

Compared to the crude odds ratios, the adjusted effect estimates increased in most cases but did not change the significance level. For all different outcome variables the risk was higher in infants with predominant margarine consumption compared with the mixed consumption group, even though statistical significance was only reached for lifetime prevalence of symptomatic eczema and doctor-diagnosed eczema, and for allergic sensitisation against inhalant allergens. No statistically significant associations were found for butter intake and any of the outcome variables.

Table 20 Crude and adjusted associations between outcomes and exposure categories of butter and margarine consumption in the LISA-study population at the age of 2 years

Variable		Butter group (N=782)		Margarine group (N=188)	
		OR	95% CI	OR	95% CI
Eczema symptoms					
Incidence in the second year of life	OR[2]	1.33	(0.97-1.81)	1.06	(0.58-1.94)
	aOR[3]	1.29	(0.89-1.88)	1.30	(0.67-2.55)
Lifetime prevalence	OR[2]	1.06	(0.85-1.32)	1.49	(1.04-2.13)[4]
	aOR[3]	1.05	(0.80-1.37)	1.71	(1.12-2.61)[4]
Doctor-diagnosed eczema					
Incidence in the second year of life	OR[2]	1.09	(0.77-1.55)	1.35	(0.74-2.48)
	aOR[3]	1.02	(0.68-1.55)	1.70	(0.84-3.41)
Lifetime prevalence	OR[2]	1.01	(0.80-1.27)	1.77	(1.23-2.55)[4]
	aOR[3]	0.97	(0.74-1.29)	2.10	(1.36-3.25)[4]
Allergic sensitisation[1] against					
Food or inhalant allergens	OR[2]	0.96	(0.71-1.29)	1.52	(0.97-2.40)
	aOR[3]	0.98	(0.68-1.39)	1.52	(0.89-2.58)
Food allergens	OR[2]	1.00	(0.72-1.38)	1.36	(0.80-2.30)
	aOR[3]	1.03	(0.69-1.55)	1.58	(0.87-2.86)
Inhalant allergens	OR[2]	0.95	(0.60-1.52)	2.17	(1.18-4.00)[4]
	aOR[3]	1.00	(0.57-1.72)	2.10	(1.01-4.41)[4]

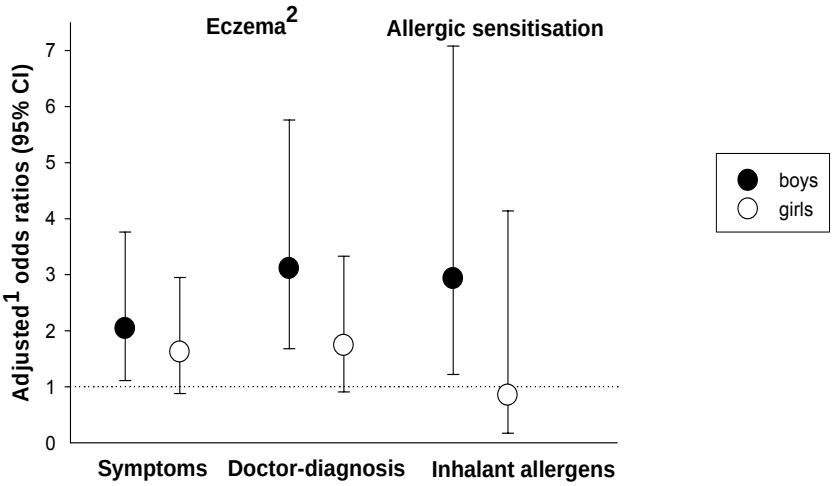
[1] IgE ≥ 0.35 kU/l; [2] crude odds ratio; [3] odds ratio adjusted for study area, gender, maternal age at delivery, maternal smoking during 2nd/3rd trimester of pregnancy, level of parental education, exclusive breast-feeding during the first 4 months, family history of atopic diseases, fresh fruit intake, salad and raw vegetable intake, keeping a dog, keeping a cat; [4] $p<0.05$

4.2.4 Sensitivity analysis

All associations, which turned out to be statistically significant in the adjusted logistic regression models, were then analysed stratified for gender, parental history of atopy and time of exclusive breastfeeding to look for potential effect modification.

Gender-stratified logistic regression analysis indicated that the risk estimates for the association between margarine intake and allergic outcomes existed in boys but not in girls (figure 12). Even though the risk for eczema was increased in both genders, statistically significant associations were found only in boys for lifetime prevalence of symptomatic eczema (aOR: 2.04; 95% CI: 1.11-3.76), doctor-diagnosed eczema (aOR: 3.11; 95% CI: 1.68-5.76) and allergic sensitisation to inhalant allergens (aOR: 2.93; 95% CI: 1.22-7.08).

Figure 12 Adjusted logistic regression results describing the association between margarine intake and eczema and allergic sensitisation in children at 2 years of age stratified for gender

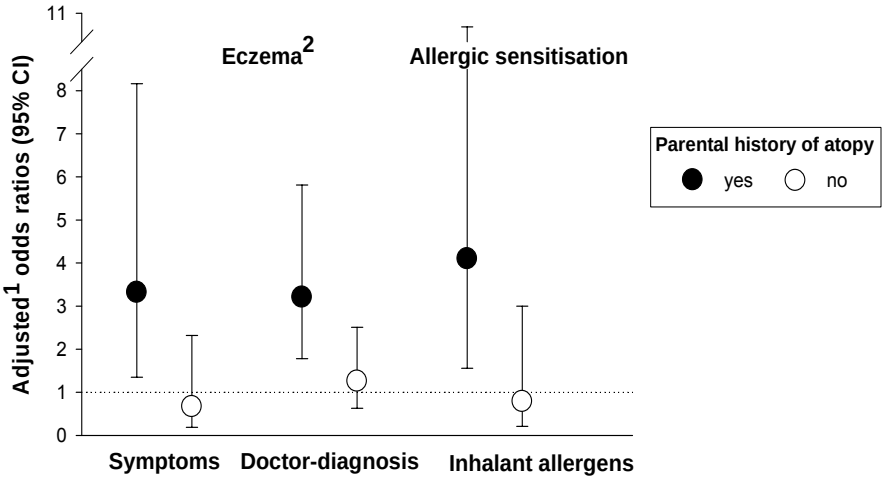


[1] adjusted for study area, maternal age at delivery, maternal smoking during 2nd/3rd trimester of pregnancy, level of parental education, exclusive breastfeeding for at least 4 months, parental history of atopy, fresh fruit intake, salad and raw vegetable intake, keeping a cat, keeping a dog; [2] Lifetime prevalence

Further stratification for parental history of atopy revealed that the increased effect estimates for the association between margarine intake and allergic outcomes are limited to children born to parents with atopic diseases (figure 13). Compared to children with a negative parental history of atopy, the risks were dramatically increased for lifetime prevalence of symptomatic eczema (aOR: 3.32; 95% CI: 1.35-8.16), doctor-diagnosed eczema (aOR: 3.21; 95% CI: 1.78-5.81) and allergic sensitisation to inhalant allergens (aOR: 4.10; 95% CI: 1.56-10.76).

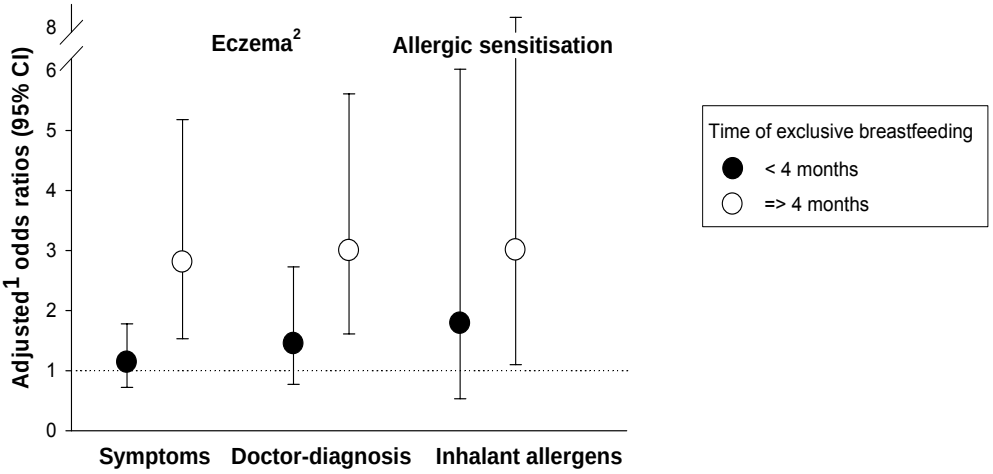
To look at potential effect modification by time of exclusive breastfeeding, a further sensitivity analysis was conducted (figure 14). Similarly, statistically significant associations were consistently limited to children who were breastfed for at least 4 months. The adjusted odds ratios for lifetime prevalence of symptomatic eczema, doctor-diagnosed eczema, and allergic sensitisation to inhalant allergens were 2.81 (95% CI: 1.53-5.18), 3.00 (95% CI: 1.61-5.61) and 3.01 (95% CI: 1.10-8.22), respectively.

Figure 13 Adjusted logistic regression results describing the association between margarine intake and eczema and allergic sensitisation in children at 2 years of age stratified for parental history of atopy



[1] adjusted for study area, gender, maternal age at delivery, maternal smoking during 2nd/3rd trimester of pregnancy, level of parental education, exclusive breastfeeding for at least 4 months, fresh fruit intake, salad and raw vegetable intake, keeping a cat, keeping a dog; [2] Lifetime prevalence

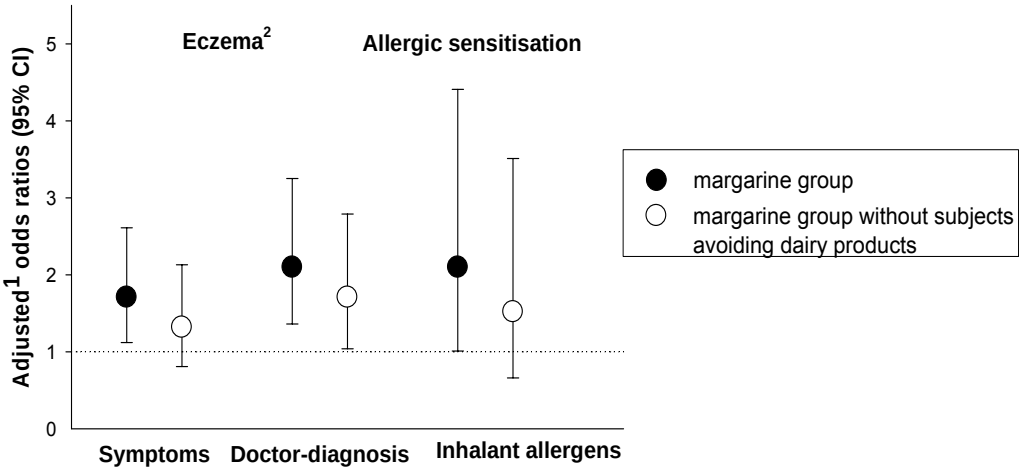
Figure 14 Adjusted logistic regression results describing the association between margarine intake and eczema and allergic sensitisation in children at 2 years of age stratified for time of exclusive breastfeeding



[1] adjusted for study area, gender, maternal age at delivery, maternal smoking during 2nd/3rd trimester of pregnancy, level of parental education, parental history of atopy, fresh fruit intake, salad and raw vegetable intake, keeping a cat, keeping a dog; [2] Lifetime prevalence

To consider a possible influence of reverse causation, we further conducted a sensitivity analysis and excluded children who deliberately avoided dairy products during the past 6 months. All effect estimates for the associations between margarine consumption and eczema and allergic sensitisation to inhalant allergens decreased, but they were still high (figure 15).

Figure 15 Adjusted logistic regression results describing the association between margarine intake and eczema and allergic sensitisation in children at 2 years of age restricted to children not avoiding dairy products



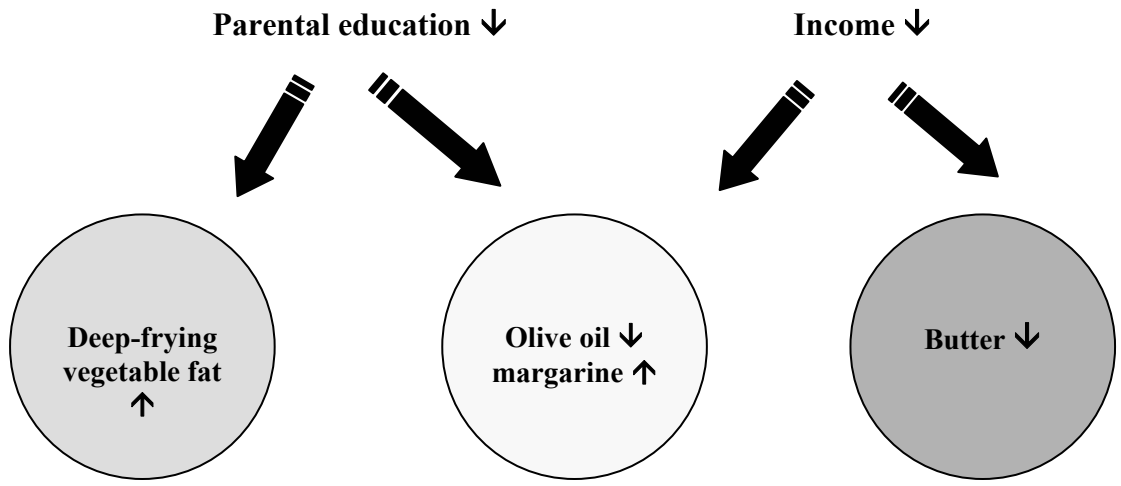
[1] adjusted for study area, maternal age at delivery, maternal smoking during 2nd/3rd trimester of pregnancy, level of parental education, exclusive breastfeeding for at least 4 months, parental history of atopy, fresh fruit intake, salad and raw vegetable intake, keeping a cat, keeping a dog; [2] Lifetime prevalence

5 DISCUSSION

5.1 Socioeconomic determinants of dietary fat intake in children

The results of this study suggest that socioeconomic factors affect the intake of dietary fats in 2-year-old children. Excepting rapeseed oil, dietary fat consumption considerably differs between the different regions in our study population. As summarised in figure 16, olive oil intake decreased with both decreasing parental education and income, while margarine intake increased. High intake of deep-frying vegetable fat was predicted by low parental education only, while butter intake was positively associated with income only.

Figure 16 Summary of the influence of parental education and income on dietary fat intake in 2-year-old children



No influence on: Sunflower oil, rapeseed oil, safflower oil

The present findings are supported by results from an adult population in France. Czernichow et al (92) assessed the intake of added oil and fat in 6572 middle-aged subjects by using 24-hour dietary records and described the relationship with region of residence and educational level. Margarine intake showed an inverse association with educational level, whereas oils with monounsaturated fatty acids were positively associated with educational level. Furthermore, they identified significant regional differences in animal fat, MUFA and PUFA intake. However, other studies provided conflicting findings. In Finland, Laitinen et al (93) collected data on dietary fat intake in 9- to 15-year-old children using the 48-hour recall method. Children of families with higher socioeconomic status consumed more margarine and less butter than did children of families with lower socioeconomic status. Similar results have

been provided by Roos et al who observed an inverse association between socioeconomic status and butter intake in a Finnish adult population of 1861 men and women (94).

Other studies mainly focused on the relationship between socioeconomic determinants and the compliance with a healthy diet. These former findings generally supported the idea that people from higher socioeconomic classes have higher intakes of healthy foods, such as fruit and vegetables, and at the same time lower intakes of foods supposed to be less healthy, such as meat and fat (95-104).

Various measures of socioeconomic status such as education (95;96;98;99;101;103;104), occupation (98;99;101), and income (97;101) have been applied to investigate their association with food and nutrient intake. The majority of these studies were performed either using only one single variable as an indicator of socioeconomic status or using more socioeconomic determinants, but failing to assess the independent contribution of each indicator. Two previous investigations demonstrated that education, occupation, and income may affect food consumption in different ways due to different underlying social processes and thus do not serve as adequate proxies for one another (105;106). Therefore, they highlighted the need for multiple indicator approaches coupled with simultaneous adjustment so that independent associations with food intake emerge. The independence of socioeconomic indicators was also confirmed by results of a large survey carried out in Germany that showed relatively weak correlations between income and education and between income and occupation, respectively (107). Therefore, present study assessed the independent contribution of parental education and equivalent income on children's dietary fat intake.

Limitations

Several limitations of our analysis need to be considered. First, children's intake of butter, margarine, vegetable oils and deep-frying vegetable fat were assessed in terms of fat used for meal preparation at home. We cannot prove that household consumption frequencies are a valid surrogate for the intake in 2-year-old children in general, but assume that even if children do not consume all meals prepared at home, it is unlikely that their own meal is prepared with other fats than the reported ones. Second, parents of participating children have reached a comparatively high level of education. In 2002, the Federal Statistical Office in Germany estimated that 31% (108) of all adults between 20 and 39 years have completed more than 10^{th} grade according to the German educational system. Thus, in our study more than twice as many subjects reached high levels of education compared to the total German

population. Therefore, it is likely that we have under-represented children from lower social classes, even if we consider the urban overrepresentation of our study population.

In this context, it also has to be taken into account that the socioeconomic variables used in our analysis are not completely independent. In figure 17 and figure 18, respectively, the proportions of children with different levels of parental education and equivalent income according to the study areas are illustrated. Higher proportions of children with high levels of parental education were observed in Munich (79.4%) compared to Wesel/Bad Honnef (60.7%) and Leipzig (53.1%). A similar pattern appeared for equivalent income subject to study area. Munich (49.1%) showed the highest proportion of children with high levels of equivalent income, followed by Wesel/Bad Honnef (23.7%) and Leipzig (16.5%).

Figure 17 Proportion of children with low, medium and high parental education in the different study areas

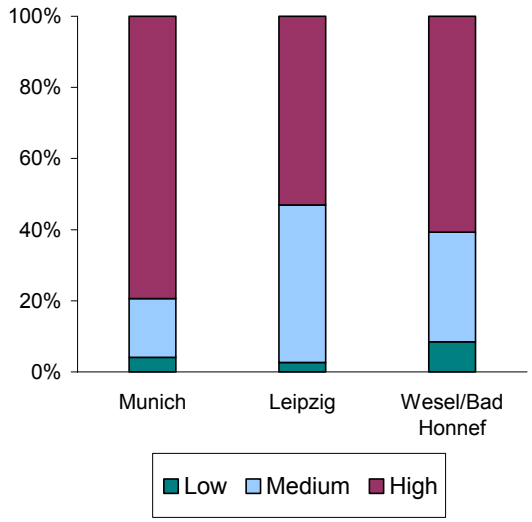
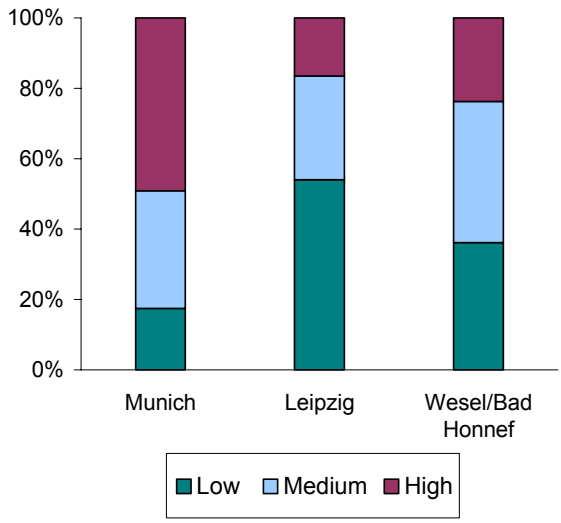
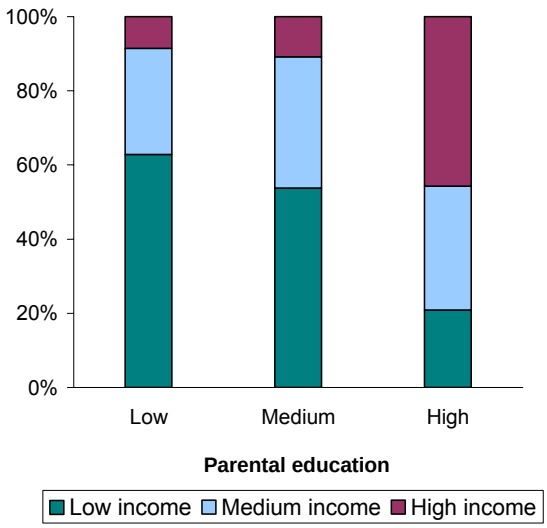


Figure 18 Proportion of children with low, medium and high equivalent income in the different study areas



Furthermore, we investigated the association between parental education and equivalent income. High equivalent income was mainly found in the group of high parental education (45.7%), while there were no major income differences between medium (high equivalent income 10.9%) and low (high equivalent income 8.6%) levels of parental education (figure 19).

Figure 19 Proportion of children with low, medium and high equivalent income according to level of parental education



To minimise the effect of correlation between socioeconomic indicators, we simultaneously adjusted for study area, parental education and equivalent income.

Moreover, we investigated how often dietary fats were used, but did not consider portion sizes. As a result, children were assigned to different intake categories based on food consumption frequencies only, which might have led to some misclassification. Furthermore, the food frequency method highly depends on the participant's ability to recall usual consumption frequencies of specific foods during the last 6 months. Since recall-ability has been shown to differ between socioeconomic groups (109), we were unable to determine whether the same degree of validity was achieved in each socioeconomic group. Some previous studies also considered the fact that over-reporting of healthy foods mainly occurs among subjects with higher levels of education, as they have a greater knowledge about healthy diet and therefore might tend to overstate their true consumption (99;100). This would in fact introduce some bias. However, in our opinion it cannot completely explain the variation in dietary fat intake by level of parental education seen in our analysis.

Strengths

One strength of this study is due to its large sample size. Previous investigations with comparable study designs have mainly analysed data of less children than we did. More importantly, studies dealing with nutrition-related issues in early childhood are in general scarce, particularly those concerning the association between social determinants and dietary fat intake. This is also one of the first studies trying to assess the independent contribution of various measures of socioeconomic position to dietary intake in children. As far as we know,

the association between income and intake of single food items in children has never been investigated before.

5.2 Maternal dietary fat intake during pregnancy and allergic diseases in the offspring

The results of this prospective study suggest that maternal dietary fat intake during the last 4 weeks of pregnancy has an effect on the development of allergic diseases in the offspring. In particular, high maternal intakes of margarine, vegetable oils and deep-frying vegetable fat were positively associated with either eczema or allergic sensitisation in children at 2 years of age. In turn, high fish consumption during the last 4 weeks of pregnancy seemed to decrease the risk of eczema in childhood.

These findings are consistent with the hypothesis that the development of allergic diseases in childhood can be affected by intrauterine exposure to factors modulating foetal immune responses (110). Margarine and vegetable oils with a high content of n-6 PUFAs (36) might be responsible for the observed positive association between maternal intake of these fats and eczema in their offspring. This assumption is supported by the results of several studies reporting that margarine intake has an adverse effect on allergic diseases in children and adults (61;73;74;111). In turn, there is evidence for a protective effect of fish intake on asthma and other allergic diseases (64;66;67), possibly because of the anti-inflammatory properties of n-3 PUFAs contained in oily fish.

Only a small number of studies have investigated the effect of maternal dietary fat intake during pregnancy on the development of allergic diseases in the offspring. In an Italian cross-sectional study involving 988 children and adolescents, Calvani et al (112) studied differences in maternal consumption of fish, butter and margarine during pregnancy in relation to allergic sensitisation in the offspring. Information on maternal intake during pregnancy was retrospectively assessed at the time of enrolment in the study. Allergic sensitisation was determined by skin prick test (SPT) to inhalant and food allergens. Frequent maternal fish intake during pregnancy was associated with a substantially reduced risk of food sensitisation in the offspring of mothers who did not suffer from allergic diseases. However, there was no association in the group of allergic mothers. Although this study has some major limitations due to the retrospective dietary assessment and the different ages when skin prick tests were performed, these finding provide some further evidence for a beneficial effect of fish intake on allergic manifestations. In our study population, we could not detect any association

between maternal fish intake during pregnancy and allergic sensitisation against food allergens. Allergic sensitisation determined by SPT has been demonstrated to differ from the assessment by measuring allergen-specific IgE in serum (113), which makes it difficult to compare our finding with the findings by Calvani et al. Furthermore, they did not report any association between margarine or butter intake and allergic sensitisation. The authors suggested that the intake of margarine and butter in the study population was probably to low to identify any potential effect.

In a nested case-control study including 691 schoolchildren, maternal fish consumption during pregnancy was assessed by retrospective telephone interviews. Maternal intake of oily fish during pregnancy was significantly associated with a reduced asthma risk in childhood, but only in children born to asthmatic mothers (114). Although recall of diet over a long period may be inaccurate, these results are in accordance with our observations.

Some studies investigated the effect of supplementation with n-3 PUFAs during pregnancy on immune responses in children at high risk of atopy. Dunstan et al (115) conducted a randomised placebo-controlled clinical trial including 83 atopic pregnant women. Subjects in the intervention group received fish oil capsules containing n-3 PUFAs from 20 week gestation. Neonates whose mothers took fish oil supplements during pregnancy had significantly lower IL-13 concentrations in their cord blood than did the control group. Follow-up of the study further showed that infants in the intervention group were less likely to be sensitised to egg allergen and had significantly less severe atopic dermatitis at 1 year of age (116).

Limitations

Several possible limitations of the study need to be considered. Misclassification of dietary exposure is always a major issue in epidemiologic studies. We applied a semiquantitative FFQ, which provided qualitative information on maternal consumption frequencies during pregnancy, but no details on usual serving sizes were collected. However, a body of evidence supports the idea that the increment of additional quantitative information on diet is extremely small (117). This has been attributed to the fact that frequency explains most of the variation in total food intake, and that intraindividual variability in serving sizes is generally greater than interindividual variability. Anyway, random misclassification of exposure would tend to bias effect estimates towards the null value. Therefore, misclassification bias is unlikely to affect significant associations.

Furthermore, we cannot completely rule out, that the reported associations were modified by the diet of the children during the first 2 years of life. We estimated the correlation between maternal consumption frequencies of fish during pregnancy and the time of introduction of fish to the infant's diet during the first year of life. The correlation coefficients did not indicate a statistically significant association, but it might be possible that maternal food consumption frequencies are a surrogate marker of infant food consumption frequencies during the first 2 years of life. This would indicate that our findings might be at least partly a consequence of postnatal dietary influences. However, interventional trials are necessary to disentangle maternal diet from the diet of the child. Reverse causation is a further potential source of bias. Reverse causation might be a problem, if atopic mothers alter their diet during pregnancy in order to prevent the onset of allergic diseases in their offspring. However, no systematic differences in the effects of maternal dietary fat intake were found between children with and children without parental atopy. Furthermore, simultaneous adjustment for all dietary variables in the multiple logistic regression models might raise concern of overadjustment. We did not observe any substantial differences between the crude and adjusted models. Thus, it is unlikely that overadjustment affected our findings. Because of a lack of statistical power, this study could not provide valid data to identify more specific associations between single foods and specific food sensitisation.

Strengths

One of the major strengths of the present study was the large sample size, which enabled us to detect statistically significant associations, which are biologically plausible but have not been described previously. Furthermore, this is the first epidemiological study estimating the association between maternal dietary fat intake during pregnancy and allergic sensitisation assessed by measuring specific IgE antibodies in serum. A prospective study design, as used in the present study, is certainly superior to a cross-sectional design for studying cause-effect relationships.

5.3 Butter and margarine intake and allergic diseases

This study suggests a positive association between margarine intake and eczema and allergic sensitisation in children at the age of 2 years. These results are consistent with previous observational studies in children. We found no association between butter intake and eczema or allergic sensitisation.

In a study investigating time trends in East Germany between 1991/92 and 1995/96, von Mutius et al found a positive association between increased margarine consumption and the

prevalence of hay fever, and an inverse relationship between increased butter intake and hay fever and atopic sensitisation among schoolchildren at the age of 9-11 years (14). Another cross-sectional survey conducted in Germany compared 5-14 year old children with respect to the type of fat they used as spread. Boys with exclusive margarine consumption had a higher risk for allergic sensitisation and rhinitis symptoms compared to those with exclusive butter consumption. No associations were found for either doctor-diagnosed hay fever, asthma or allergic diseases in girls (73). In Finland, Dunder et al employed a 48-h recall to assess dietary fat intake in children aged 3-18 years. They analysed the data in two different ways. In view of the longitudinal data, they could show that children, who developed atopic disease during the follow-up of 9 years, had consumed less butter before the outbreak of atopic disease. According to the cross-sectional data, children with atopic diseases consumed more margarine and less butter than non-atopic children did (67).

Two studies on respiratory symptoms in children estimated dietary margarine and butter intake by means of a semi-quantitative FFQ. In an Italian study, consumption of bread with margarine was associated with wheeze and consumption of bread with butter with shortness of breath with wheeze (76). A Dutch birth cohort study found an inverse association between daily butter consumption and asthma and wheeze in pre-school children (75). Further evidence for both the increased risk of margarine consumption (61;74) and the protective effect of butter consumption (72) is published by studies on asthma in adults. In our study, there was no clear evidence for any association between butter consumption and eczema and allergic sensitisation.

In search of a biological mechanism, mainly two hypotheses have been discussed. Comparing the fatty acid composition of margarine and butter, margarine contains 10 to almost 30 times more linoleic acid than butter (36). This could partly explain the negative impact of margarine and the neutral effect of butter on allergic diseases in our study population. Besides n-6 PUFAs, trans fatty acids have been linked to an increased prevalence of allergic diseases (118). In Germany, the content of trans fatty acids in vegetable oil margarine (2.8%), dietetic margarine (0.4%) and margarine semi-fat (0.8%) is not higher than in ordinary butter (2.8%) (36) and has been strongly decreased during the past years (119). At the same time linkage with food consumption data revealed that butter contributes about 50% to the total intake of trans fatty acids in German households (120). Therefore, it is not obvious why margarine consumption, but not butter consumption should increase the risk for atopic diseases when considering trans fatty acids.

Stratified multiple logistic regression analysis revealed that increased risks for eczema and allergic sensitisation with high margarine intake were mainly attributed to children with a positive parental history of atopic diseases. This observation might indicate a genetic contribution to differences in fatty acid metabolism potentially involved in atopy. Strong associations between the fatty acid composition in serum phospholipids and variants in the genes encoding delta-5 and delta-6 desaturase, which are known to be the key enzymes in the metabolism of polyunsaturated fatty acids, have been recently identified (121). Although only a weak association between this gene cluster and hay fever was found in this study, other authors suggested linkage of the chromosomal region, where this gene cluster is located, and atopy (122). If genetically determined differences in fatty acid metabolism between atopic and nonatopic subjects exist, this might explain why the effects of margarine intake on eczema and allergic sensitisation have occurred in subjects with positive parental history of atopy only. This hypothesis is also supported by observations indicating that patients with atopic diseases, especially eczema (123), have altered serum fatty acid compositions (124). Abnormal fatty acid compositions were also seen in the cord blood of newborns at high risk of atopic diseases (125).

Genetic influence might also explain the results of epidemiological studies on effect modification by gender. As previously reported (73), the associations in our study population between margarine consumption and allergic disease in gender-stratified analysis were mainly limited to boys. In a different context, effects of polymorphisms involved in fat metabolism have been shown to depend on gender (126). In this way, gender-specific effects of genotype on fatty acid metabolism could be responsible for the higher susceptibility to allergic diseases in boys. In any case, the results of the stratified analysis have to be interpreted with great caution due to small numbers in each stratum and as formal testing for interaction did not show significant differences between boys and girls.

Limitations

There are several important aspects when interpreting the findings of this study. First, we did not have adequate data to prove the hypothesis that n-6 PUFA intake is causally associated with the development of allergic diseases. Therefore, we used margarine intake as a proxy for n-6 PUFA intake. Since it has been shown that in addition to fats and vegetable oils more products contribute to the total intake of PUFAs (120), other sources of n-6 PUFA should have been taken into account. Furthermore, we could not consider brand-related differences in the fatty acid composition of butter and margarine. Thus, we cannot be sure, that margarine consumption is a valid surrogate for n-6 PUFA intake, but assume that the dietary exposure

used in present study can at least explain a high proportion of the variability in n-6 PUFA intake. In addition, total spread intake in children at age 2 years is probably not very high. Therefore, we combined consumption frequencies of cooking and baking at home with the spread intake of the child in one exposure variable. Assuming that household consumption frequencies can be used as a surrogate for the diet of the child, this provides a more precise insight into the actual intake of margarine and butter.

Furthermore, we cannot completely rule out that reverse causation has biased our findings. A recent recommendation suggested an oligo-allergenic diet, if food allergy is suspected in patients with eczema. This diet also includes milk-free margarine (127). In our study population, children with predominant margarine consumption reported to avoid dairy products almost twice as much as children in the butter group did (14.4% vs. 7.9%). Since effect estimates in multivariate analysis were lowered when excluding those who deliberately avoided dairy products, the association between predominant margarine consumption and eczema could also reflect altered dietary patterns due to the diagnosis. A second sensitivity analysis showed that parents with atopic diseases (8.3%) more frequently indicated to leave dairy products out of the child's diet than parents without atopic diseases (4.2%). Thus, children with allergic diseases might consume more margarine than healthy children, because atopic parents have restricted the diet of their child to prevent or to delay the outbreak of allergic diseases. This is also indirectly supported by the observation that children who were breastfed for at least 4 months have higher risks of a positive outcome than children who were breastfed for less than 4 months. As breastfeeding is related to a health-conscious behaviour this might indicate that parents with atopic diseases more likely breastfed their child and more likely avoid dairy products but use margarine instead.

In this context, the temporal sequence between exposure and outcome should be thoroughly ascertained. Effect estimates for lifetime prevalence of eczema were consistently higher than effect estimates for incidence rates in the second year of life, indicating that children with predominant margarine consumption have a higher risk to develop eczema in the first year of life than in the second year. However, information on butter and margarine consumption was gathered for the age 1.5 to 2 years. This leads to the suggestion that not margarine intake in children alone completely explains the increased risk for eczema. Assuming that the diet of children reflects the diet of their parents, a prenatal influence of the mother's diet has to be taken into consideration due to the fact that we showed margarine to be stronger related to early onset of eczema. However, only 16% of all children whose mothers reported a high

margarine intake during pregnancy predominantly consumed margarine and 78% consumed both butter and margarine.

Further discussion is related to the discrepancy we observed between reported symptoms of eczema and doctor-diagnosed eczema that has already been described before. A Swedish study demonstrated that the diagnosis of atopic dermatitis based on parental evaluation could not be verified by dermatologists in one-third of the cases (128). The reason why this discrepancy was not existent within children consuming margarine and more pronounced in children consuming butter in our study could not be finally clarified. As we have shown that parents of children in the butter group have reached the highest educational levels, we suspect that these parents are more health conscious than parents in the margarine group are. They might report eczema symptoms more likely and thus, in reality symptoms may be more frequent in the margarine group than those subjects evaluate.

We further cannot completely rule out that residual confounding by other lifestyle factors has biased our findings. Smoking is one of the key determinants of health (101), and was strongly related to margarine intake in our study population. Compared to mothers in the butter group, almost twice as many mothers of children in the margarine group were smoking during pregnancy. Furthermore, it is well documented that subjects with lower socioeconomic status consume a less healthy diet, particularly less fruits and vegetables (104;129;130). In our study population, children of parents with low levels of education less likely consumed salad and raw vegetables and at the same time more likely consumed margarine than children of higher educated parents did. This indicates complex associations among different lifestyle factors that might not have been sufficiently considered by adjustment in the analyses.

5.4 Methodological differences between the analyses

The association between dietary fat intake and eczema and allergic sensitisation has been analysed in this work both prospectively and cross-sectionally. The idea to link maternal diet during pregnancy with allergic diseases in children trace back to the hypothesis suggesting that many chronic diseases have their origin in prenatal and postnatal development (131-133). A prospective approach to identify dietary risk factors seems therefore superior to a cross-sectional approach. However, findings from cross-sectional studies can give important information on associations, when dietary exposure reflects a habitual intake over a large period of time. When comparing the results of the analyses presented in this work, these methodological differences should be kept in mind.

Furthermore, different approaches to create dietary variables from the questionnaire-derived information on food consumption frequencies were used. The choice of the method was depending on the particular objective of the analysis and the distribution of food intake in the study population. In the first analysis presented in this work, the intention was to look at extremely low and extremely high consumption of dietary fats in relation to socioeconomic factors. The lowest and the highest quintile of intake reflect extremely high and extremely low consumption frequencies, respectively. Whether the lowest or the highest quintile for one fat was analysed in relation to the exposure variables, was dependent on the intake distribution of the particular fat. If fats were almost never consumed by a large part of the study population, subjects with a high intake of this specific dietary fat were of primary interest and the other way round. The second analysis was rather driven by the underlying biological hypothesis. A high maternal intake of foods rich in certain fatty acids was therefore analysed in relation to allergic diseases in the offspring. For this purpose, the highest tertile, or alternatively the upper two tertiles, were compared with the lower two tertiles or alternatively, the lowest tertile. Again, the decision whether the highest tertile or the upper two tertiles were compared with the reference group was based on the intake distribution of the food item.

The method of using quantiles as cut-off point to create dichotomous variables has one major advantage over using cut-off points based on an arbitrarily selected consumption frequency. In this way, differences in the general consumption frequency of the food are considered and similarly strong occupied reference categories are provided.

The approach applied in the third analysis was aiming at differences in butter and margarine consumption patterns. Mutually exclusive exposure variables were therefore created to study high margarine and high butter intake separately compared with a dietary pattern of either consuming both margarine and butter or none of them.

5.5 Comparison of the results

A direct comparison between the effect of maternal dietary fat intake and dietary fat intake in children is limited due to the methodological differences discussed in chapter 5.4.

Margarine intake was positively associated with the prevalence of doctor-diagnosed eczema in 2-year-old children, both in terms of maternal intake during the last 4 weeks of pregnancy and in terms of children's intake. However, when stratifying these effects, boys were more likely affected by eczema when they had a high margarine intake themselves. In contrast, if mothers had a high margarine intake during pregnancy, girls were more likely suffering from

eczema during the first 2 years of life than boys. Previous studies predominantly reported stronger effects in male than in female subjects (57;61;73), but the reason for this is not clarified yet.

Further discrepancies between the two analyses were observed in respect of effect modification by parental atopy and exclusive breastfeeding. Children who consumed margarine had a higher risk for eczema in case their parents had atopic diseases and they were exclusively breastfed for at least 4 months. However, no effect modification by parental atopy or exclusive breastfeeding could be shown for the association between maternal diet and eczema in the offspring. In the light of the observation that these effects were limited to the cross-sectional analysis, this might give reason to the assumption that reverse causality has biased our findings. This would be a result of parents with atopic diseases who deliberately alter the diet of their child with intent to prevent the outbreak of atopic diseases. If the child then develops allergic diseases, the observed association between margarine intake and eczema would be a statistical artefact. Furthermore, differences in the size of the dietary exposure groups between the two analyses should be considered. While only 7.3% of all children belonged to the margarine group, the group of children with a high maternal margarine intake during pregnancy counted 33.4% of all subjects. Stratification further reduces the number of subjects in each stratum. Therefore, the analysis on maternal diet on eczema certainly has more power to detect statistically significant associations which might give a possible explanation for the differences in the results.

Another inconsistency is due to the effect of margarine intake on allergic sensitisation against inhalant allergens. First of all, under the assumption that linoleic acid intake leads to an increased IgE production, it is not clear why margarine intake is associated with increased specific IgE levels against inhalant but not against food allergens. Second, the findings that maternal intake of margarine during pregnancy is associated with eczema but not with sensitisation might raise some doubt about the reliability of these findings. In this context, it should be at least mentioned that other mechanisms of PUFAs than increased PGE_2 and subsequent IgE production might stimulate inflammatory processes as illustrated in the introduction. These processes could play a role in eczema but not in clinically inapparent sensitisation. Anyway, future studies are warranted to investigate the effect of margarine consumption on allergic sensitisation and allergic diseases, to better elucidate the precise mechanism.

Butter intake did not show any association with eczema or allergic sensitisation, neither in term of maternal intake nor children's intake. All other dietary fats, such as vegetable oils, deep-frying vegetable fat and fish have been assessed in maternal diet only and thus no comparison is possible.

5.6 Conclusions

Socioeconomic factors were found to have an impact on dietary fat intake in children. Regional differences in dietary fat intake appeared not only between the East and the West, but also between study areas in the North and in the South and between urban and more rural areas. These regional differences might be a consequence of disparities in education and income but also of geographical and cultural differences across the study areas. The influence of education and income further seemed to be independent from each other and its nature to depend on the type of fat. However, the results of this study are heterogeneous and thus it is too early to draw a final conclusion by now.

The results of this study also provide some evidence for an association between dietary fat intake and eczema in young children. Maternal margarine intake during pregnancy as well as margarine intake during childhood was a strong risk factor for eczema in children at 2 years of age. This is in line with previous studies reporting positive associations between margarine intake and allergic diseases in children and adults. Butter intake did not show any statistically significant effect in this study, similar to recently published studies. Furthermore, maternal intake of vegetable oils during pregnancy was positively associated with eczema during the first two years of life, whereas fish intake had a protective effect. These observations are consistent with suggestions that n-6 PUFAs might promote and n-3 PUFAs might reduce the risk for allergic diseases. However, we cannot prove that these associations are due to a causal role of dietary components and not confounded by other dietary or lifestyle factors.

Allergic sensitisation against inhalant allergens at 2 years of age was positively associated with maternal intake of deep-frying vegetable fat during pregnancy and margarine intake during childhood. Due to the low prevalence of sensitisation against inhalant allergens at this age and inconsistencies between the study results, these findings should be interpreted with caution.

There was also some indication for gender-specific effects of dietary fat intake on allergic manifestations, although these effects were not consistent across the analyses and not statistically significant when formally tested for interaction. Previous studies predominantly

reported stronger effects in male than in female subjects, but the reason for these observations is not clear. This highlights the need for further investigation in this subject.

All results presented in this thesis only cover the first 2 years of life. Due to the age dependency of allergic diseases and sensitisation, it would be worthwhile to see whether the effects of prenatal and early postnatal diet on allergic manifestations persist into later childhood or even beyond.

Currently, no recommendations are being made to mothers or to children to modify their diets for the prevention of allergic diseases because of insufficient evidence of a beneficial effect. Before any recommendations can be made, randomised clinical intervention trials should be performed to confirm the cause-effect relation observed in the present study.

6 SUMMARY

Dietary fatty acid intake has been proposed to influence the development of allergic diseases. In this context, polyunsaturated fatty acids, n-6 and n-3 fatty acids, are mainly discussed. N-6 fatty acids can be metabolised by the organism to inflammatory eicosanoids playing a role in allergic inflammation and promoting the production of immunoglobulin E. In contrast, eicosanoids synthesised by n-3 fatty acids have several times less anti-inflammatory properties. This led to the hypothesis that the ratio of n-6 to n-3 fatty acids in the diet plays a role in the development of allergies.

For the analyses presented in this thesis, data from the prospective birth cohort study LISA ('Influences of **L**ifestyle related Factors on the **I**mmune **S**ystem and the Development of **A**llergies in Childhood') were used. Between November 1997 and January 1999, 3097 newborns were recruited from obstetrical clinics in the four German cities Munich, Leipzig, Wesel, and Bad Honnef. Shortly after childbirth, questionnaires on family history of atopy, socioeconomic status, smoking during pregnancy and maternal diet during pregnancy were administered to the parents. Data on the children's health, their diet and different lifestyle factors were collected by using repeated parental-completed questionnaires at regular time intervals during the follow-up period (at 6, 12, 18, and 24 months of age). In addition, blood samples for total and specific immunoglobulin E analysis were drawn at 2 years of age.

Maternal diet during the last 4 weeks of pregnancy was assessed by using a semiquantitative food-frequency questionnaire that was answered shortly after childbirth. To assess dietary fat intake in children at the age of 2 years, on the one hand parents were asked how often they have used butter, margarine, vegetable oils and other fats for cooking, baking, frying and preparing salad dressings at home. Furthermore, the children's individual intake of butter and margarine as spread was reported. This information was combined and three groups of children with a) predominant butter intake, b) predominant margarine intake and c) intake of butter and margarine or intake of none of both were formed.

Symptomatic eczema was defined as itchy rash that was not restricted to the skin underneath the diaper, and that was either recurrent or persisted more than 14 days. Doctor-diagnosed eczema was based on a positive answer to the question: "Has a doctor diagnosed your child with allergic or atopic eczema in the past 6 months?" Allergic sensitisation was defined as a specific serum IgE concentration $\geq$0.35 kU/l. Thereby, allergic sensitisation against food allergens was differentiated from sensitisation against inhalant allergens. Multiple logistic

regression analyses were applied to estimate associations between dietary fat intake and eczema and allergic sensitisation taking potential confounders into account.

The results of this study showed that dietary fat intake in 2-year-old children is influenced by region, level of parental education and income level in different ways.

Furthermore, maternal dietary fat intake during the last 4 weeks of pregnancy had an effect on the development of allergic diseases in the offspring. In particular, high maternal intake of margarine and vegetable oils during pregnancy was positively associated with doctor-diagnosed eczema in children during the first 2 years of life, whereas high maternal fish consumption seemed to decrease the risk of childhood eczema. In turn, high maternal intake of deep-frying vegetable fat significantly increased the children's risk to become sensitised against inhalant allergens but not against food allergens. Maternal butter intake during pregnancy was not related to any of the outcomes.

A positive association was observed between margarine intake and the prevalence of eczema and allergic sensitisation in children at the age of 2 years. Children with predominant margarine intake also had more than a twofold increased risk for being sensitised against inhalant allergens but not against food allergens. Stratified analysis further revealed that effects tend to be stronger in boys than in girls and stronger in children with atopic parents compared to those with nonatopic parents. Associations were also more pronounced in children who were exclusively breastfed for at least 4 months compared to those who were breastfed for less than 4 months. No associations were found between butter intake and eczema or allergic sensitisation.

The results of this study suggest that dietary fat intake is associated with eczema and allergic sensitisation in children. In particular the association between maternal diet during pregnancy and later development of allergic diseases in the offspring has rarely been investigated yet. Therefore, this study contributes to the scientific knowledge in this field. However, whether these observations are causal, cannot finally be determined in this study and should therefore be verified in future studies.

7 ZUSAMMENFASSUNG

Es wird vermutet, dass der Verzehr von bestimmten Fettsäuren die Entstehung allergischer Erkrankungen beeinflussen kann. Insbesondere mehrfach ungesättigte Fettsäuren, n-6 und n-3 Fettsäuren, scheinen dabei eine Rolle zu spielen. N-6 Fettsäuren können im Organismus zu entzündlichen Eicosanoiden metabolisiert werden, die an der allergischen Entzündung beteiligt sind und die Bildung von Immunglobulin E fördern. Dahingegen besitzen Eicosanoide, die aus n-3 Fettsäuren synthetisiert werden, um ein Vielfaches geringere inflammatorische Eigenschaften. Dies führte zu der Hypothese, dass das Verhältnis von n-6 zu n-3 Fettsäuren in der Ernährung eine Bedeutung in der Entstehung von Allergien haben könnte.

In der vorliegenden Arbeit wurden Daten der prospektiven Geburtskohortenstudie LISA (‚Einfluss von **L**ebensstilfaktoren auf das **I**mmun**S**ystem und die Entwicklung von **A**llergien bei Kindern') analysiert. Dazu wurden im Zeitraum von November 1997 bis Januar 1999 3097 Neugeborene in den vier deutschen Städten München, Leipzig, Wesel und Bad Honnef rekrutiert. Kurz nach der Geburt füllten die Eltern Fragebögen zu atopischer Familienanamnese, sozioökonomischem Status, Rauchen während der Schwangerschaft und der Ernährung der Mutter während der Schwangerschaft aus. Informationen über die Gesundheit des Kindes, dessen Ernährung und unterschiedliche Lebensstilfaktoren wurden in regelmäßigen Zeitabständen während des Follow-ups (im Alter von 6, 12, 18 und 24 Monaten) gesammelt. Zusätzlich wurden im Alter von 2 Jahren Blutproben für die Analyse von Gesamt- und spezifischem Immunglobulin E genommen.

Die Ernährung der Mutter während der vier letzten Wochen der Schwangerschaft wurde mit Hilfe eines semiquantitativen Verzehrshäufigkeitsfragebogens erhoben, der zum Zeitpunkt der Geburt ausgefüllt wurde. Um den Fettverzehr der Kinder im Alter von 2 Jahren zu ermitteln, wurden einerseits die Eltern befragt, wie häufig sie Butter, Margarine, pflanzliche Öle und andere Fette zum Kochen, Backen, Braten und für die Zubereitung von Salatdressings verwenden. Zudem wurde der individuelle Verzehr der Kinder von Butter und Margarine als Brotaufstrich abgefragt. Diese Informationen wurden kombiniert und drei Gruppen von Kindern gebildet, die a) überwiegend Butter, b) überwiegend Margarine oder c) etwa gleich viel Butter und Margarine oder aber keines von beiden verzehren.

Ekzemsymptome wurden definiert als juckender Hautausschlag, der nicht auf den Windelbereich beschränkt war und wiederholt auftrat oder länger als 14 Tage andauerte. Die ärztliche Diagnose eines Ekzems beruhte auf einer positiven Beantwortung der Frage: „Hat

ein Arzt bei Ihrem Kind in den letzten 6 Monaten Neurodermitis, ein allergisches oder atopisches Ekzem festgestellt?“ Eine allergische Sensibilisierung wurde durch das Vorhandensein von spezifischen IgE-Konzentrationen von ≥0.35 kU/l definiert. Dabei wurde zwischen der Sensibilisierung gegenüber Nahrungsmittelallergenen und der Sensibilisierung gegenüber Inhalationsallergenen differenziert. Um den Zusammenhang zwischen Fettzufuhr und Ekzem und allergischer Sensibilisierung zu untersuchen, wurden multiple logistische Regressionsmodelle gerechnet.

Die Ergebnisse dieser Arbeit zeigen, dass die Fettzufuhr von 2-jährigen Kindern stark von der Region, der elterlichen Bildung und des Einkommens abhängig ist, diese Einflüsse jedoch nicht klar voneinander abzugrenzen sind.

Es konnte des Weiteren ein Zusammenhang zwischen der Fettzufuhr der Mutter während der letzten 4 Wochen der Schwangerschaft und der Entstehung von allergischen Erkrankungen bei Kindern beobachtet werden. Eine hohe Zufuhr von Margarine und pflanzlichen Ölen war positiv mit der ärztlichen Diagnose eines Ekzems während der ersten beiden Lebensjahre assoziiert, wohingegen ein hoher Fischkonsum das Risiko zu mindern schien. Das Risiko einer allergischen Sensibilisierung gegen Inhalationsallergene, nicht jedoch gegen Nahrungsmittelallergene, stand in positivem Zusammenhang mit der Zufuhr von pflanzlichen Kochfetten. Der Verzehr von Butter während der Schwangerschaft war mit keinem der Endpunkte assoziiert.

Bei Kindern im Alter von 2 Jahren wurde eine positive Assoziation zwischen dem Verzehr von Margarine und dem Auftreten von Ekzem und allergischer Sensibilisierung nachgewiesen. Kinder, die überwiegend Margarine verzehrten, hatten zudem ein mehr als zweifach erhöhtes Risiko, gegen Inhalationsallergene sensibilisiert zu sein. In der stratifizierten Analyse zeigte sich für alle Effekte tendenziell eine stärkere Assoziation für Jungen im Vergleich mit Mädchen. Ähnliche Unterschiede ergaben sich in den Effekten zwischen Kindern mit und Kindern ohne atopische Familienanamnese und zwischen Kindern, die mehr als 4 Monate ausschließlich gestillt wurden und denjenigen, die kürzer gestillt wurden. Butterverzehr war mit keinem der Endpunkte assoziiert.

Die Ergebnisse der vorliegenden Arbeit lassen einen Zusammenhang zwischen Fettverzehr und dem Auftreten von Ekzem und allergischer Sensibilisierung bei Kindern vermuten. Besonders der Zusammenhang zwischen der mütterlichen Ernährung während der Schwangerschaft und der Entstehung von allergischen Erkrankungen bei Kindern wurde bisher nur wenig untersucht. Deshalb liefert diese Arbeit einen interessanten Beitrag zu den

bisherigen Erkenntnissen auf diesem Gebiet. Ob diese Beobachtungen jedoch kausaler Natur sind, kann nicht abschließend beurteilt werden und sollte deshalb in zukünftigen Studien verifiziert werden.

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Hiermit erkläre ich, Stefanie Sausenthaler, dass ich die vorliegende Dissertation selbständig angefertigt habe. Ich habe mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft und Bezeichnung der Fundstelle einzeln nachgewiesen. Ich habe bisher noch keinen Promotionsversuch unternommen, und die vorliegende Dissertation wurde nicht in gleicher oder ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht.

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Stefanie Sausenthaler

Curriculum Vitae

PERSÖNLICHE DATEN:

Name:	Stefanie Sausenthaler
Geburtsdatum/-ort:	24.02.1980 in Fürstenfeldbruck

AUSBILDUNG:

11/1999 – 08/2004	Studium der Ökotrophologie an der Technischen Universität München Abschluss: Dipl. Oec. Troph. Univ.
02/2004 – 08/2004	Diplomarbeit an der TU München in Zusammenarbeit mit dem Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt, Institut für Epidemiologie, Neuherberg Thema: „Kochsalzzufuhr und bronchiale Hyperreagibilität (ECRHS I)“
seit 09/2004	Doktorarbeit an der Ludwig-Maximilians-Universität München im Fach Humanbiologie Thema: „Socioeconomic determinants of dietary fat intake and the effect of dietary fat intake on allergic diseases in children“
10/2004	Intensivkurs „Clinical Epidemiology” an der Medizinischen Fakultät der Ludwig-Maximilians-Universität München
08/2006	Summer School „Ernährungsepidemiologie“ am Deutschen Institut für Ernährungsforschung, Potsdam-Rehbrücke

BERUFLICHE TÄTIGKEITEN:

09/2004 – 04/2007	Doktorandin am Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt, Institut für Epidemiologie, Neuherberg
seit 05/2007	Wissenschaftliche Mitarbeiterin am Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt, Institut für Epidemiologie, Neuherberg

PUBLIKATIONEN:

Sausenthaler S, Kompauer I, Brasche S, Linseisen J, Heinrich J. Sodium intake and bronchial hyperresponsiveness in adults. Respir Med 2005; 99:864-70.

Sausenthaler S, Heinrich J. Geschmacksprägung in der frühen Kindheit. Kinderärztliche Praxis 2006; in: „Kinderernährung und gesunder Lebensstil“; 77: 32-34.

Sausenthaler S, Kohlhammer Y, Schäffer L, Koletzko S, Koletzko B, Heinrich J. Genetically determined lower bitter-taste sensitivity in Africans? (Letter). Br J Nutr 2006; 96: 607-608.

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Sausenthaler S, Kompauer I, Borte M, Herbarth O, Schaaf B, Berg v A, Zutavern A, Heinrich J for the LISA Study Group. Margarine and butter consumption, eczema and allergic sensitization in children. The LISA birth cohort study. Pediatr Allergy Immunol 2006; 17: 85-93.

Sausenthaler S, Kompauer I, Mielck A, Borte M, Herbarth O, Schaaf B, Berg v A, Wichmann HE, Heinrich J for the LISA Study Group. Impact of parental education and income inequality on children’s food intake. Public Health Nutrition 2007; 10:24-33.

Sausenthaler S, Koletzko S, Schaaf B, Lehmann I, Borte M, Herbarth O, Berg v A, Wichmann HE, Heinrich J for the LISA Study Group. Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 years of age. Am J Clin Nutr 2007; 85:530-7.