Early life determinants of atopy

A 20-year prospective follow-up study on unselected, healthy newborns

Maria Pesonen

Academic dissertation

To be presented with the permission of the Faculty of Medicine, University of Helsinki, for public examination in the auditorium of Skin and Allergy Hospital, Meilahdentie 2, Helsinki, on April 11th 2008, at 12 noon.
To my family
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by Roman numerals (I-IV).


ABBREVIATIONS

ApoB-100  apolipoprotein B 100
ApoE      apolipoprotein E
CCR       chemokine receptor
CI        confidence interval
COX       cyclo-oxygenase
CS-IgE    cord serum immunoglobulin E
DC        dendritic cells
DGLA      dihomo-gamma-linolenic acid
DHA       docosahexaenoic acid
EPA       eicosapentaenoic acid
FHA       family history of allergy
Foxp3     forkhead/winged helix transcription factor
GALT      gut-associated lymphoid tissue
GLA       gamma-linolenic acid
HDL       high-density lipoprotein
IDL       intermediate-density lipoprotein
IFN-γ     interferon gamma
IgA       immunoglobulin A
IgE       immunoglobulin E
IgG       immunoglobulin G
IgM       immunoglobulin M
IL        interleukin
LDL       low-density lipoprotein
LOX       lipoxygenase
LR        likelihood ratio
LT        leukotriene
MCP-1     monocyte chemotactic protein 1
n-3       omega-3
n-6       omega-6
OR        odds ratio
PG        prostaglandin
PPV       positive predictive value
SD        standard deviation
SE        sensitivity
SEM       standard error of mean
sIL-4R    soluble IL-4 receptor
SP        specificity
SPT       skin prick test
TGF-β     transforming growth factor beta
Th1       type 1 helper T cell
Th2       type 2 helper T cell
Th3       type 3 helper T cell
TLR       toll-like receptor
TNF       tumor necrosis factor
Tr1       type 1 regulatory T cell
VA        verified atopy
VLDL      very low-density lipoprotein
ABSTRACT

Atopy-related allergic diseases, i.e. allergic rhinoconjunctivitis, atopic dermatitis and asthma, have increased in frequency in the industrialized countries during the past decades. Such a rapid increase may not be solely due to genetic factors. Instead, the changes in lifestyle associated with increasing affluence, such as improved hygiene, urbanization and alterations in the dietary habits, are thought to be responsible for the rapid increase of the prevalence of allergic diseases.

In order to reverse the trend of increasing allergies, effective allergy-preventive strategies need to be developed. This requires a better understanding of the early-life events leading to the expression of the atopic phenotype. Therefore, the present study has aimed at defining early-life factors and markers associated with the subsequent development of allergic diseases in a long-term follow-up cohort of Finnish newborns.

The study cohort consists of 200 unselected, healthy newborns prospectively followed up from birth to age 20 years. Their mothers were encouraged to start and to maintain exclusive breastfeeding as long as it was nutritionally sufficient for the infant. Consequently, all the infants received some duration of exclusive breastfeeding, 58% of the infants were on exclusive breastfeeding for the first 6 months of life, and 18% received exclusive breastfeeding for the first 9 months or above. A total of 94% of the infants were on exclusive or partial breastfeeding at 2 months of age, and the similar percentage was 74% and 58% at ages of 6 and 9 months, respectively. Of the infants, 42% had a family history of allergy. Cord serum IgE, total cholesterol and plasma retinol were measured, and the cholesterol and retinol concentrations were repeatedly measured during the first year of life. The children were re-assessed at ages 5 (n=163), 11 (n=150) and 20 years (n=164) with clinical examination, skin prick testing, blood sampling and parental and personal structured interviews.

Exclusive breastfeeding prolonged for 9 months or above was associated with atopic dermatitis and symptoms of food hypersensitivity at age 5 years, and with symptoms of food hypersensitivity at age 11 years, in children with a family history of allergy. Cord serum immunoglobulin E (IgE) concentration of 0.5 kU/L or above was associated with subsequent atopic manifestations in childhood and up to age 20 years. The sensitivity of cord serum IgE in predicting skin prick test-verified atopy at ages of 5 and 20 years was 50% and 26%, respectively. A combination of an elevated cord serum IgE and a positive family history of allergy predicted atopic manifestations with a higher positive likelihood ratio and specificity than the cord serum IgE level or the family history of allergy alone. Retinol concentration at 2 months of age was inversely associated with positive skin prick test at ages of 5 and 20 years, and with allergic symptoms at age 20 years. Children and adolescents with allergic symptoms, skin prick test positivity and an elevated IgE had lower total cholesterol levels in infancy and childhood than the nonatopic subjects. The differences in cholesterol and
retinol levels of the infants with and without subsequent atopy were not detectable in cord blood, but became significant from age 2 months onward.

In conclusion, prolonging strictly exclusive breastfeeding for over 9 months of age was not helpful in prevention of allergic symptoms, instead, it was associated with increased atopic dermatitis and food hypersensitivity symptoms in childhood. Due to the modest sensitivity, measurement of cord serum IgE cannot be recommended as an exclusive screening method for atopic predisposition; however, by using the combination of family history of allergy and an elevated cord serum IgE as a criterion, it is possible to identify a group of infants who are very likely to develop allergic symptoms later in life. Retinol concentration in young infants is inversely associated with the subsequent development of allergic symptoms. The inverse association between the cholesterol level in infancy and subsequent clinical and laboratory manifestations of atopy may not be due to atopy-related dietary alterations, since it was already present in early infancy, when virtually all the infants were on a similar diet, i.e. on human milk feeding. Based on these findings, it is proposed that there may be differences in the inborn regulation of retinol and cholesterol levels in children with and without a genetic susceptibility to atopy, and these may play a role in the development of atopic sensitization and allergic diseases.
INTRODUCTION

The development of allergic diseases, i.e. allergic asthma, atopic dermatitis, allergic rhinoconjunctivitis and food allergies, is dependent on the interaction of genetic and environmental risk factors. The genetic background provides susceptibility to produce IgE antibodies against common environmental allergens and to develop allergic diseases. However, the rapid increase in the prevalence of allergic diseases experienced in industrialized countries during the past few decades may not be explained solely by genetic factors. Instead, the changes in lifestyle such as improved hygiene, urbanization and alterations in the dietary habits are thought to have contributed to the increased prevalence of allergy.

It has been suggested that improved hygiene and public health measures of the industrialized countries have reduced and modified the exposure to infectious and environmental microbes, which otherwise might stimulate the immune system and favor immune responses that protect from the development of allergic diseases. The development of allergic diseases is thought to have its origins in early infancy, in a period when the newborn's inexperienced immune system is maturing to develop an effective protection against pathogens and to achieve immunological tolerance towards harmless antigens. Certain environmental factors, to which children are exposed early in life, may influence the developing immune system and possibly contribute to the development of allergic immune response in a genetically susceptible individual.

In order to develop effective allergy-preventive strategies, a more detailed understanding of the pathogenesis of allergic diseases is required. The aim of the present study is to identify early-life factors and markers associated with subsequent development of allergic diseases, and to clarify the effect of exclusive breastfeeding on allergy development in a cohort of unselected healthy Finnish newborns followed-up from birth to 20 years of age.
REVIEW OF THE LITERATURE

Definitions of atopy and allergic diseases

Atopic predisposition is a genetic tendency to develop allergic diseases, i.e. allergic asthma, allergic rhinoconjunctivitis and atopic dermatitis, and to produce immunoglobulin E (IgE) antibodies in response to common environmental allergens such as food antigens, animal dandruff, tree and grass pollen and house dust mite (Johansson 2001).

Atopic manifestations include the objective signs of atopic sensitization (skin prick test positivity, allergen-specific serum IgE positivity, elevated serum total IgE) as well as the clinical symptoms of allergic diseases.

Atopic dermatitis, i.e. atopic eczema, is a chronic, relapsing, itching, inflammatory skin disease with typical distribution and morphology (Hanifin 1980, Leung 2000).

Allergic rhinitis is a combination of nasal symptoms such as sneezing, itchy rhinitis and nasal congestion, which are periodical and unrelated to infection (Skoner 2001). Allergic rhinoconjunctivitis is defined as the presence of nasal symptoms accompanied with itchy and watery eyes on aeroallergen exposure.

Food hypersensitivity is defined as an adverse reaction to food causing objectively reproducible symptoms or signs at a dose tolerated by normal subjects. Food hypersensitivity reactions in which immunologic mechanisms are demonstrated are referred to as food allergy. Immunologic reactions to food in which an IgE-mediated mechanism is established are defined as IgE-mediated food allergy (Johansson 2001).

Asthma is defined as an inflammatory airway disease marked by increased numbers of bronchial inflammatory cells such as mast cells and eosinophilic cells, and manifesting as recurrent, reversible symptoms of bronchial obstruction (Martinez 1998, Haastela 1996).
Prevalence of allergic diseases

The prevalence of allergic diseases, i.e. asthma, allergic rhinoconjunctivitis, atopic eczema and food allergy as well as the prevalence of allergen-specific IgE antibodies has increased in developed countries during the past decades (Taylor 1984, Burney 1990, Chulada 2003, Linneberg 2000, Sampson 2004, Kosunen 2002, Latvala 2005, Law 2005, Gupta 2007, World Health Organization 2002). In a worldwide study carried out in 1990s, atopic diseases in childhood were found to be considerably more prevalent in industrialized than in developing countries (ISAAC Steering Committee 1998).

In Finland, the prevalences of asthma, allergic rhinoconjunctivitis and atopic eczema in a total of 11607 children aged 13-14 years from four geographic regions were examined in 1994-5 as a part of the ISAAC study. The reported prevalences of atopic diseases were as follows: allergic rhinoconjunctivitis ever 44-55%, allergic rhinoconjunctivitis during the past year 15-23%, eczema ever 23-26%, flexural dermatitis during the past year 15-19%, asthma ever 4-8%, and wheezing during the past year 13-20% (Remes 1998, Pekkanen 1997). Previously, lower prevalences of atopic diseases were reported from south-western Finland in adolescents aged 15-16 years; among them, the prevalence of doctor-diagnosed asthma was 2.5%, and the prevalences of a history of allergic rhinitis and atopic dermatitis were 14% and 9.7%, respectively (Varjonen 1992).

A feature of the atopic manifestations is the characteristic sequence of the onset of the symptoms, referred to as the “atopic march”. Atopic dermatitis and food allergies typically are the first symptoms to appear in infancy, followed by asthma and allergic rhinitis later in childhood (Bergmann 1994, Kulig 1999, Rhodes 2002). However, only a minority of infants with atopic eczema goes on to develop asthma later in childhood (van der Hulst 2007). Asthmatic wheezing may be observed in early infancy, though the majority of the children with early wheezing turn out to be transiently symptomatic, and only a minority develops a persisting atopic asthma (Martinez 1995). Infantile eczema may precede and predict subsequent development of allergic sensitization in some infants, whereas in others, the allergic sensitization may precede and predict the development of eczema (Lowe 2007). The first IgE responses to food proteins, particularly to hen’s egg and cow’s milk, may be observed during the first months of life (Kulig 1999). Sensitization to indoor and outdoor environmental allergens requires more time and is generally observed between the first and tenth year of life (Wahn 2001, Kulig 1999). Strong IgE responses to food proteins in infancy have been shown to predict subsequent sensitization to aeroallergens (Nickel 1997). Seasonal allergic rhinoconjunctivitis is generally not observed during the first 2 years of life, although a minority of children has specific IgE antibodies to aeroallergens during this early period (Bergmann 1994, Kulig 1999).
Factors contributing to the development of allergic diseases and sensitization

Family history of allergy

An inherited predisposition, together with multiple environmental contributing factors, triggers the development of allergic immune responses. Family history of allergy is the strongest known predictor of atopic diseases (Tariq 1998). In a Swedish study, the cumulative prevalence of these diseases in children aged 7-14 years was found to be 38% in children with single parental history of atopy and 52% in those with a biparental history of atopy, whereas it was 18% in children without a parental history of atopy (Åberg 1993). According to a prospective follow-up study, the cumulative incidence of atopic dermatitis was 38% in children with a single parental allergic history, and 50% in those with a biparental history (Böhme 2003).

Within the last few years, several allergy and asthma susceptibility genes such as ADAM33, PHF11 and GPRA have been identified (Van Eerdewegh 2002, Jang 2005, Laitinen 2004) and numerous gene polymorphisms, such as the toll-like receptor 4 (TLR4) and the lipopolysaccharide receptor CD14 polymorphisms, have been identified that are associated with atopic manifestations (Ober 2006, Kim 2007, Eder 2004, Fagerås-Böttcher 2004). The loss-of-function mutations of filaggrin, an epidermal barrier protein, have been recognized as predisposing factors for atopic eczema (Palmer 2006). Filaggrin deficiency leads into impaired skin barrier function, which allows increased entry of allergens and other antigens into the skin. The transcutaneous allergen exposure may lead to allergic sensitization, which has been suggested to contribute to the development of asthma when the allergens, to which the immune system has been primed by cutaneous exposure, enter the airways (Irvine 2006). It has been estimated that half of the children with moderate to severe atopic eczema may carry filaggrin gene mutations (Irvine 2006). Nevertheless, the genetics may not solely account for the rapid increase in the prevalence of allergic diseases that have occurred in affluent countries during the past few decades. Instead, changes in the living environment and subsequent gene-environment interactions are thought to be responsible for the change (Marks 2006).
Environmental factors

Reduced exposure to microbes

The increased prevalence of atopic diseases in affluent countries is thought to be attributable to a reduced microbial burden in childhood, which is a consequence of “westernized”, modern lifestyle with improved hygiene, diminished family size and urbanization. The hypothesis that a reduced amount of infectious diseases in childhood might be associated with increased prevalence of atopic diseases was originally presented in 1976 (Gerrard 1976). Later, this theory became known as the “hygiene hypothesis” (Strachan 1989, von Mutius 1994 and 2000). Children with several older siblings as well as those attending day-care during infancy have been found to have a reduced risk of allergic sensitization, hay fever and asthma later in life (Strachan 1996, Farooqi 1998, Krämer 1999, Ball 2000). This is thought to be due to early and frequent exposure to infectious agents (Wahn 2001).

Children growing up on a farm, i.e. in an environment rich in microbial components, have a significantly lower prevalence of asthma, hay fever and allergic sensitization, and the effect has been shown to persist into adulthood (Ernst 2000, Riedler 2001, Remes 2003, Eduard 2004, Radon 2004). Furthermore, a very early or even prenatal exposure to farm environment with livestock was associated with a strongly reduced risk of atopy, wheeze and asthma in a cross-sectional European study (Ege 2006). Exposure to foodborne and orofecal microbes (hepatitis A virus, Toxoplasma gondii and Helicobacter pylori) has been inversely associated to respiratory allergy in a case-control study on Italian young men (Matricardi 2000). The authors suggest that seropositivity to these microbes may be a marker of being reared in an environment that provides a high exposure to many other orofecal and foodborne microbes, which may have atopy-preventive effects.

In newborn mammals, the proper maturation of the gastrointestinal tract and the immune system is dependent on the presence of the commensal bacteria in the gut (Hooper 2004, Rhee 2004). The gut immune response, induced by microbial antigens, is assumed to play an important role in the postnatal maturing the peripheral immune response (Macpherson 2004, Noverr 2004, Mazmanian 2005). Therefore, the quality of the microbial flora colonizing the gut may affect the development and priming of the immune system in infancy. Children from Estonia, a country with a low prevalence of atopy, have been shown to have a different intestinal bacterial flora including more lactobacilli and less clostridia than children from Sweden, where the prevalence of atopy is high (Sepp 1997). Furthermore, allergic children have been shown to be less often colonized with lactobacilli and to have higher counts of intestinal aerobic microbes than the nonallergic children (Björksten 1999). Consistent with this, the children who have been born on farms and exposed to raw,
unpasteurized milk with a high microbial load and particularly lactobacilli, have a low prevalence of allergy and asthma (von Ehrenstein 2000, Waser 2007). The anthroposophic lifestyle, characterized by a diet including fermented vegetables which are rich in lactobacilli, is also associated with a reduced prevalence of atopy (Alm 1999). In placebo-controlled trials, administration of probiotic preparations to pregnant mothers and their infants for the first 6 months of life have been shown to reduce the incidence of atopic eczema up to the ages of 2 and 7 years. However, in these studies, the probiotic treatment did not have an effect on the incidence of sensitization or allergic diseases other than atopic eczema (Kalliomäki 2001 b, Kukkonen 2006, Kalliomäki 2007).

Early exposure to indoor allergens

Even though indoor allergen avoidance is an important measure in established asthma and sensitization, it does not seem to be very effective in the primary prevention (Corver 2006). In a German longitudinal birth cohort study, a dose-response relationship between early exposure to indoor allergens (cat and house dust mite) and the risk of sensitization during the first 3 years of life was demonstrated (Wahn 1997). However, the early indoor allergen exposure was shown to be unrelated with the prevalence of asthma, wheeze and bronchial hyperresponsiveness at the age of 7 years (Lau 2000). Exposure to high concentrations of mite allergens in early infancy have been reported to be a risk factor for developing atopic dermatitis during the first 3 years of life (Huang 2001). According to a Norwegian study, early life exposure to pets may be associated with a reduced risk of developing allergic diseases, mainly atopic eczema (Nafstad 2001). Another possible interpretation of this finding is that keeping pets is a marker of a lifestyle that protects against allergic diseases.

Tobacco smoke exposure

Maternal smoking increases the infant’s susceptibility to infections (DiFiranza 2004, Jedrychowski 1997) and is associated with the development of asthma and allergy in the infant (Martinez 1988, Magnusson 1986). Maternal smoking during pregnancy affects the fetal IgE production (Magnusson 1986), lymphoproliferative and cytokine responses (Devereux 2002, Noakes 2003), and inhibits neonatal Toll-like receptor mediated innate immune responses, which are thought to promote the regulatory pathways in the inhibition of allergic immune responses (Noakes 2006).
Dietary factors

Mode of infant feeding

Breastfeeding is the preferred source of nutrition for the newborns, including infants at high risk as well as those with a low risk of allergic disease (Friedman 2006). If supplementation to breastfeeding is needed, avoidance of cow's milk based formula during the first days of life may reduce the risk of cow's milk allergy (Saarinen 1999). If breastfeeding is not possible, use of hydrolyzed milk formula instead of a conventional cow's milk formula has been suggested to diminish the risk of sensitization to cow's milk in infants with a high hereditary risk of allergy (Osborn 2003).

According to a recent study on infants with a family history of allergy, use of hydrolyzed formula instead of cow's milk formula as a supplementation of breastfeeding may reduce the risk of atopic dermatitis up to age 3 years (von Berg 2007). However, the Finnish guideline for management of food allergy in children does not recommend the use of hydrolyzed supplementary formulae as a means of primary prevention of cow's milk allergy (Current Care Guideline, Duodecim 2004). This is in line with a recent literature review, which concludes that none of the published studies have shown the so-called hypoallergenic formulae to be effective in allergy prevention (Brand 2007).

Maternal diet during pregnancy and lactation

Maternal dietary allergen avoidance during pregnancy has been shown ineffective in preventing allergic diseases in the infant (Fälth-Magnusson 1992). However, increased maternal intake of vitamin E, vitamin D and zinc during pregnancy may be related to a reduced risk of asthma, atopic sensitization and eczema in early childhood (Litonjua 2006, Devereux 2006, Devereux 2007). A high maternal consumption of fish, rich in n-3 polyunsaturated fatty acids, during pregnancy may be associated with a reduced risk of atopic eczema and asthma in childhood (Sausenthaler 2007, Dunstan 2003, Salam 2005). According to some studies, the maternal avoidance of dietary allergens during lactation may diminish the risk of atopic dermatitis and food allergies in early childhood (Hattevig 1989, Zeiger 1995). However, the protective effect did not last later in childhood (Zeiger 1995, Hattevig 1999), and some studies could not demonstrate any allergy-protective effect of a maternal avoidance diet during lactation (Herrmann 1996, Pollard 1996).
Dietary polyunsaturated fatty acids

The dietary habits have been markedly altered in the developed countries during the past decades. The consumption of saturated fat of animal origin has decreased and that of vegetable-derived n-6 polyunsaturated fatty acids has increased, whereas the consumption of n-3 polyunsaturated fatty acids, derived mainly from oily fish, has decreased (Sanders 2000). In industrialized countries, the ratio of n-6 and n-3 fatty acids in the diet has risen from the former 1-2:1 to a ratio of 15:1 (Simopoulos 2006).

The essential polyunsaturated fatty acids are precursors of eicosanoids, such as prostaglandins and leukotrienes. The eicosanoids synthesized as a result of n-6 polyunsaturated fatty acid metabolism are considered proinflammatory, whereas the metabolism of the n-3 fatty acids leads to formation of less inflammatory eicosanoids and to a competitive inhibition of the n-6 fatty acid metabolism. For this reason, the alterations in dietary fat intake are considered as possible contributing causes for the increase in allergic diseases (Black 1997, Porkka 1997). In support of this, several studies have reported that the consumption of margarine and vegetable oils rich in n-6 polyunsaturated fatty acids is associated with allergic diseases (Dunder 2001, Wakai 2001, Solvoll 2000, Sausenthaler 2006).

Breastfeeding

Breastfeeding as the preferred method of feeding a newborn

Human milk, with its unique and dynamically changing protein and lipid composition, is considered to optimally meet the infant's nutrient requirements during the first 6 months of life (Lönnerdal 2003, Koletzko 2001). Breastfeeding has been shown to protect the infant against infectious diseases (Bhandari 2003, Bachrach 2003, Duncan 1993), obesity (Grummer-Strawn 2004) and several chronic illnesses such as type 1 diabetes mellitus and inflammatory bowel disease (Virtanen 2003, Corrao 1998). Breastmilk feeding also has beneficial effects on lipoprotein profile and blood pressure in adolescence (Singhal 2004). The benefits of exclusive breastfeeding have been documented in iron and cholesterol nutrition (Siimes 1984, Kallio 1992).

Exclusive breastfeeding during the first 4-6 months of life is widely recommended (American Academy of Pediatrics 1997, Department of Health and Social Security 1994, Kramer and Kakuma 2001, Hasunen 2004). However, according to a recent study, Finnish infants received exclusive breastfeeding for a median duration of 1,4 months only. Of the infants, 58% were exclusively breast-fed at age 1 month, 22% at age 4 months, and only 1% at age 6 months (Erkkola 2006).
Breastfeeding in allergy prevention

Based on systematic literature reviews, exclusive breastfeeding for up to 4 months of age is considered protective against childhood asthma and atopic eczema, especially in children with a family history of allergy (Gdalevich 2001 a, Gdalevich 2001 b, van Odijk 2003). However, studies on the effects of breastfeeding on the development of allergic sensitization and clinical atopy have yielded controversial results, some studies indicating an allergy-preventive effect (Saarinen 1979 and 1995, Oddy 1999 and 2004), while other studies have failed to confirm such an effect (Taylor 1983 and 1984, Sears 2002, Miyake 2003, Kramer 2007). A prospective Finnish follow-up study concluded that breastfeeding is prophylactic against atopic disease including atopic eczema, food allergy, and respiratory allergy, throughout childhood and adolescence (Saarinen 1995). According to an Australian prospective birth cohort study, exclusive breastfeeding was associated with a reduced risk of asthma and aeroallergen sensitization at age 6 years; the risk of asthma was reduced by 4% with each additional month of exclusive breastfeeding (Oddy 1999 and 2004). In a large cluster-randomized trial conducted in Belarus, an intervention promoting breastfeeding decreased the risk of atopic eczema during the first year of life (Kramer 2001). However, a follow-up study conducted in Germany indicates that prolonging non-exclusive breastfeeding may increase the risk of atopic eczema during the first 7 years of life (Bergmann 2002). In a follow-up study on infants small for gestational age, breastfeeding was associated with an increased risk of atopic eczema at age 3,5 years (Purvis 2005). Based on a cross-sectional study, it was concluded that breastfeeding may be associated with increased atopic eczema at age 12-15 years (Miyake 2003).

The effect of breastfeeding on the risk of atopic dermatitis may be dependent on whether the child has a family history of allergy. In a follow-up study on Finnish children, exclusive breastfeeding for 3 months was protective against atopic dermatitis at age 4 years in children with a parental history of allergy, whereas it was associated with an increased risk of atopic dermatitis at age 4 years in children without parental history of allergy (Siltanen 2003). A similar dual effect, i.e. increased risk of atopic dermatitis at age 18 months in the exclusively breast-fed infants without parental history of allergy, and a moderate protective effect in infants with a parental history of allergy, was reported from a large Danish follow-up study (Benn 2004).

According to some studies, the allergy-protective effect of breastfeeding is limited to early childhood, long-term follow-up indicating increased atopic symptoms among the breast-fed subjects (Wright 1989 and 2001). Wright et al. found exclusive breastfeeding for 4 months to protect against early recurrent wheezing, whereas it was associated with an increased risk of asthma at age 6 years in the offspring of asthmatic mothers. A similar result, i.e. increased asthma at age 5-6 years in the breast-fed children of asthmatic mothers, was reported by Oberle et al. (Oberle 2001).
**Immunological properties of human milk**

The suggested allergy-preventing effect of exclusive breastfeeding may be due to a reduced exposure to dietary antigens as well as to the effects of the immunologically active factors in human milk. In addition to meeting the infant's energy and nutrient needs, the human milk provides an immunological support system extending from the mother to the infant during the first months of life (Walker 2004). The wide variety of protective factors in human milk may compensate for the immaturity and naïveté of the infant's immune system and the low production of the defensive agents in mucosal secretions of the newborn infant (Newburg 2005, Goldman 1998).

Human milk contains antimicrobial (i.e. immunoglobulin (Ig) A, IgG, IgM, lactoferrin, lysozyme, antiviral lipids and oligosaccharides) and anti-inflammatory substances (i.e. interleukin (IL)-10, transforming growth factor (TGF)-β, IgA, antioxidants such as vitamins A, C, and E), hormones and growth factors promoting the gut maturation (Cummings and Thompson 2002). In addition, human milk contains a wide variety of immunomodulating agents including leukocytes, cytokines (i.e. interferon (IFN)-γ, tumor necrosis factor (TNF)-α) and chemokines, soluble cytokine receptors and receptor antagonists (Hamosh 2001). Oligosaccharides and other prebiotic substances in human milk promote the colonization of the gut of the newborn with bifidobacteria and lactobacillus (Walker 2004).

Human milk contains small amounts of allergens, such as dietary protein antigens (Kilshaw 1985). Recently, it was shown in mice that antigens transferred from the lactating mother to the neonate through milk may induce tolerance and protect from allergic airway disease (Verhasselt 2008). The composition of human milk shows considerable inter- and intra-individual variability (Rodriquez-Palmero 1999). Differences in the leukocyte, cytokine and chemokine composition of milk from atopic and nonatopic mothers have been observed (Järvinen 2002, Böttcher 2000 a, b). The eosinophil cationic protein in human milk has been associated with cow’s milk allergy and atopic dermatitis in the infant, even though the presence of eosinophil cationic protein in breast milk was not dependent of the maternal atopic status (Österlund 2004).

**Effect of breastfeeding and weaning on the maturation of the gut**

During breastfeeding, the small intestine of the infant is growing rapidly. However, the mucosal architecture resembles that of a neonatal intestine with narrow finger-like villi and small crypts. Weaning, the gradual process of replacement of milk feeding with an increasing range of ingested nutrients, is associated with expansion of the mucosal surface area by villous and crypt hyperplasia (Cummings 2002). The expansion of the mucosal surface by crypt hyperplasia has been suggested to be promoted by a physiological inflammation of the gut associated lymphoid tissue due to antigenic stimulation of weaning (Cummings 1997). In animal studies, the exposure to food proteins after weaning has been shown to provide an important
physiological stimulus for the maturation of the gut immune system, without which
the gut-associated lymphoid tissue (GALT) remains poorly developed and a T helper
(Th)2-dominated cytokine profile persists (da Silva Menezes 2003).

Immunological basis of the hygiene hypothesis

Balance between the T helper type 1 and 2 mediated responses

Allergic diseases are the result of a T helper type 2 (Th2) cell-mediated immune
response, which is directed against innocuous environmental antigens, i.e. allergens.
The cytokines produced by the Th2 cells, such as interleukin (IL) -4, IL-5, IL-9, and
IL-13, have an important role in the initiation, maintenance and amplification of the
allergic inflammation and asthma (Romagnani 2000, Larché 2003, Sperger 2003, Li
1999). These cytokines promote effector mechanisms characteristic for allergic
immune reaction, such as the IgE production by B cells and the eosinophil
proliferation, differentiation, recruitment and survival (Busse 2003, Farrar 2002). The
Th2 responses are essential in clearing helminth infections (Zaiss 2006).

The T helper 1 (Th1) cells stimulate the recruitment and activity of macrophages
and are involved in the cellular immunity activated by microbial antigens, typically
resulting in the production of IgG antibodies (Mosmann 1989). The cytokines
characteristic to Th1 responses are IL-12, IL-2, TNF-β and IFN-γ. The Th1 and Th2
responses have counterregulatory effects on each other, i.e. the IFN-γ produced by the
Th1 cells antagonizes the development of Th2 cells and inhibits their activities,
whereas IL-4 inhibits the development of Th1 cells (Romagnani 2006).

A reduced exposure to pathogenic and nonpathogenic microbes in childhood is
hypothesized to result in a missing immune deviation from Th2 response to Th1
response, caused by a reduced production of Th1 –polarizing cytokines by cells of the
innate immunity, i.e. dendritic cells (DC), natural killer T cells and neutrophils, in
response to stimulation of their Toll-like receptors by microbial components
(Romagnani 2004, Prescott 2005). DC play a critical role in programming the T cell
responses, and animal studies suggest that resting DC stimulate Th2 immune
development unless they receive Th1-trophic signals during antigen processing
(Stumbles 1998). Exposure to the lipopolysaccharide (LPS) of Gram-negative
bacteria, leading to Toll-like receptor 4-mediated activation, before the allergic
responses are established may prevent allergic sensitization (Tulic 2000). While low
levels of LPS appear to favour Th2 differentiation, high doses favour Th1
differentiation. Once activated via Toll-like receptor stimulation, the DC and other
antigen presenting cells show enhanced expression of costimulatory molecules and
cytokines which promote Th1 responses (Pasare 2003).
Epidemiological evidence in favour and against the immune deviation hypothesis

The observations that patients with rheumatoid arthritis or multiple sclerosis, which are Th1-polarized autoimmune diseases, exhibit a lower prevalence of allergic diseases (Hilliquin 2000, Tang 1998, Tremlett 2002) and that a preexisting asthma is associated with a decreased risk of autoimmune disorders (Tirosh 2006) support the concept of the mutual exclusivity of Th1 and Th2 responses. However, the prevalence of certain Th1-mediated autoimmune diseases (insulin-dependent diabetes mellitus, inflammatory bowel disease, multiple sclerosis) has been reported to have increased in the developed countries at the same time as that of the Th2-driven allergic conditions (Bach 2002). In addition, there is evidence that Th1 autoimmune diseases and Th2 mediated atopic diseases may coexist in the same patient (Simpson 2002). Individuals with parasitic helminth infections characteristically have Th2-polarized immune responses, but they may also have reduced allergic responses (Yazdanbakhsh 2002). Therefore, it has been suggested that the Th1 and Th2 responses may not be mutually exclusive in humans, and the immune deviation promoted by the environmental risk factors may not be a polarization of the entire immune system towards either Th1 or Th2 dominance, but instead, a deviation of the regulation of both the Th1 and Th2 responses (Simpson 2002, Fallon 2007).

In addition to Th1 and Th2 cells, another subset of helper T cells, T helper 17 cells, has been recognized. The T helper 17 cells have been suggested to have a major role in the pathogenesis of some inflammatory and autoimmune disorders including rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease and allergic airway hypersensitivity (Romagnani 2006, Hue 2006, Hellings 2003).

Regulatory T cells

The regulatory T cells are defined as specialized subsets of CD4-positive T cells (Figure 1) capable of controlling destructive immune responses to pathogens and of preventing immune responses against inappropriate targets such as self-antigens or harmless external antigens (Bacchetta 2007). The CD25-positive (CD4+CD25+Foxp3+) regulatory T cells originating from the thymus are denoted as naturally occurring, whereas subsets of regulatory T cells induced in the periphery under tolerogenic conditions throughout life are named adaptive regulatory T cells. These include the IL-10 and TGF-β producing Tr1 and Th3 (i.e. Tr2) cells (Roncarolo 2006, Prescott 2005). The suppressive function of the CD4+CD25+ T cells has been found defective in allergic patients during pollen season (Ling 2005). However, conflicting results exist with functional CD4+CD25+ T cells found in most of the atopic subjects (Bellinghausen 2003).
Figure 1. The CD4 positive effector and regulatory T cell subsets and their characteristic cytokine expression. Th1, type 1 T helper cell; Th2, type 2 T helper cell; Th17, type 17 T helper cell; Treg, regulatory T cell; DC, dendritic cell; IL, interleukin; IFN-γ, interferon gamma; TNF-β, tumor necrosis factor beta; TGF-β, transforming growth factor beta. After Romagnani 2006 and Steinman 2007.

T helper type 2 immunity during pregnancy

The T helper type 2 (Th2) responses are a prerequisite for a successful pregnancy. The fetoplacental unit produces Th2 cytokines such as IL-4 and IL-5 throughout pregnancy. These protect the fetus from maternal immune rejection by downregulating the maternal Th1 activity. As a consequence, the fetus is exposed to a high concentration of Th2 and T regulatory cytokines (Warner 2002). Human fetal lymphocytes are skewed towards a Th2 cytokine profile, which is thought to be due to intra-uterine priming by placental cytokines and possibly also by transplacental allergen exposure (Warner 1996). At present, the knowledge of the role and suppressive function of T regulatory cells in atopic subjects is limited.
**T-cell responsiveness after birth**

In a newborn infant, the Th2 responses predominate (Prescott 1998). The Th1 responses then gradually mature during the first years of life (Prescott 2005, Rowe 2000). The exposure to microbial products has been suggested to be an important stimulus for the postnatal maturation of the Th1 responses, and for the reciprocal downregulation of the Th2 responses. In nonatopic infants, the Th2 responses are suppressed during the first year of life, whereas in atopic infants, they are consolidated (Prescott 1999). A decreased capacity to produce IFN-γ, a Th1 cytokine, at birth has been associated with the continuation of the fetal Th2 responses during infancy (Prescott 1999). These and similar findings may be interpreted as a consequence of a missing shift of Th2 responses to Th1 responses in an infant prone to atopic manifestations (Romagnani 2004).

**Mechanisms of immunologic tolerance**

The T cell compartment of the immune system is able to respond to a virtually infinite variety of exogenous antigens because it consists of a large repertoire of T cell clones with unique antigen receptors. These inevitably include T cell clones capable of self-antigen recognition and a potential for dangerous autoimmune reactions, which must be regulated, i.e. a self-tolerance is to be generated and maintained (Romagnani 2006). The self-reactive T cells are controlled during ontogeny within the thymus by a process of negative selection leading to apoptosis (Starr 2003). Some of the self-reactive T cells escape the negative selection, and are controlled in the peripheral lymphoid organs by other regulatory mechanisms such as T cell anergy (Powell 2006), deletion through activation-induced cell death (Chambers 2001), and immune suppression by the natural regulatory CD4+CD25+Foxp3+ T cells (Sakaguchi 2000). The extent and duration of the protective immune responses against exogenous antigens also need to be regulated so that the immune responses do not turn dangerous to the host. These regulatory mechanisms include the reciprocal inhibition of Th1 and Th2 pathways at the level of the transcription factors, cytokines and chemokines, and the functions of the adaptive T regulatory cells such as Th3, Tr1 and the adaptive CD4+CD25+Foxp3+ cells (Weiner 2001, Battaglia 2006, Chen 2003).

Even though intact food antigens routinely penetrate the gastrointestinal tract, they infrequently induce clinical symptoms because tolerance develops in most individuals. Oral feeding of an antigen induces antigen-specific immunological hyporesponsiveness termed oral tolerance (Husby 1994 and 2000). The tolerance induced by continuous oral antigen feeding of experimental animals or by sublingual immunotherapy in humans results in the suppression of allergic manifestations not only in the gastrointestinal tract but also in tissues outside it, such as the airways (Faria 2003, Ciprandi 2007). The intestinal colonization by commensal bacteria plays a vital role in the development of the host immune system and of oral tolerance.
(Mazmanian 2005, Maeda 2001, Sudo 1997). Therefore, the quality of the colonizing microbiota may be significant with regard to the development of allergic diseases. The composition of the gut microbiota differs between allergic and nonallergic infants so that colonization with bifidobacteria and enterococci is associated with a lower incidence, and colonization with clostridia is associated with a higher incidence of allergic symptoms (Björksten 1999, Kalliomäki 2001 a, Penders 2007).

**Cord serum immunoglobulin E (CS-IgE) as a predictor of atopy**

**Early markers for screening of atopic predisposition are needed**

In order to reverse the trend of increasing allergic diseases, effective allergy preventive measures are warranted (Halken 2004, Allam 2005). While the effectiveness of dietary allergen avoidance measures (Høst 1999) has been questioned (Brand 2007), some new primary and secondary allergy preventive strategies involving the administration of probiotics (Kalliomäki 2003), oral antihistamines (Warner 2001) and specific immunotherapy (Hayashi 2006) have been suggested.

To be cost-efficient, the allergy preventive measures should be directed to subjects at a high risk for developing allergic diseases. Therefore, markers for screening of atopic predisposition are needed. Identification of early markers of atopy in cord blood would offer a possibility of efficient screening of subjects at risk. Among the markers studied as possible predictors of atopy in cord blood are the Th2-related cytokines IL-4, IL-5 and IL-13, soluble immunoglobulin receptors sCD30 and sCD23, IL-4 receptor sIL-4R, Th2-related chemokines such as eotaxin and monocyte chemotactic protein 1 (MCP-1), adiponectin, eosinophil cationic protein, cytokine profile of stimulated cord blood mononuclear cells, and the cord serum total IgE (CS-IgE) level (Allam 2005, Rothenbacher 2007, Mete 2004).

**CS-IgE measurement as a screening test for subsequent atopy**

An elevated cord serum IgE (CS-IgE) level is considered to be a risk factor for the development of allergic symptoms and sensitization in children. The CS-IgE level has been shown to be associated with polymorphisms of interleukin-13, interleukin-4 and cytotoxic T-lymphocyte antigen 4 genes (Sadeghnejad 2007, Wen 2006, Chang 2004). However, studies on the value of CS-IgE in predicting the risk of atopic manifestations have yielded conflicting findings.

Several studies have reported the CS-IgE to be ineffective in predicting early atopic symptoms, i.e. those appearing during the first 2 years of life (Eiriksson 1994,
Bergmann 1997, Hansen 1992 a, Lødrup Carlsen 1999). Some studies indicate that CS-IgE above 0.7-0.8 kU/L may predict the development of atopic disease by this age (Businco 1983, Chandra 1985), and that CS-IgE ≥ 0.3 kU/L may be associated with atopic dermatitis, and atopic disease combined with elevated total IgE in children at age 18 months (Jøhnke 2006, Hansen 1992b). In high-risk infants, CS-IgE ≥ 0.5 kU/L may also be a risk factor for urticaria due to food allergy at 12 months of age (Kaan 2000).

There are only a few reports available on the value of CS-IgE in predicting atopic manifestations after early childhood. According to a Swedish study, a high CS-IgE may be a risk factor for developing atopic disease by the age of 10-11 years, even though the sensitivity of CS-IgE measurement in predicting atopic disease was low (Croner 1990). Furthermore, an elevated CS-IgE has been shown to be associated with allergic sensitization at ages 4 and 10, and with asthma at age 10 years (Sadeghnejad 2004).

Due to the different cut-off levels used for CS-IgE and the varying definitions of atopic outcomes as well as the varying length of follow-up time, the studies investigating the CS-IgE level in allergy prediction are difficult to compare. However, the sensitivities reported for CS-IgE generally are low, within the range of 12-44%, whereas the reported specificities are within 71-94% (Lilja 1991, Tariq 1999, Hide 1991, Hansen 1992a, Croner 1990, Bergmann 1997, Hansen 1993). The low sensitivity signifies that the elevated CS-IgE level identifies only a minority of the infants who subsequently will develop atopic manifestations.

Due to the low sensitivity, the CS-IgE level is considered to be inappropriate as an exclusive predictive marker for atopic diseases (Allam 2005). However, the combination of the CS-IgE level and the family history of allergy has been suggested to be useful for identifying infants at a high risk of subsequent atopy e.g. for allergy prevention studies (Croner 1990, Zeiger 1995).

**Vitamin A**

**Physiology and metabolism of vitamin A**

Vitamin A, i.e. retinol, and its derivatives are required in various biological functions, including epidermal growth and differentiation, immunity, vision, bone development, reproduction and maintenance of the gut integrity (Napoli 1999, Kastner 1995, Quadro 2000). In vitamin A deficiency, both innate and adaptive immune systems are compromised, the Th1/Th2 balance is disturbed and the T cell-dependent antibody responses are impaired (Stephensen 2004, Pasatiempo 1990, Wiedermann 1993). Vitamin A deficiency, defined as a plasma retinol concentration of less than 0.70 μmol/l or 200 μg/l (Pilch 1987), is associated with elevated morbidity and mortality

Vitamin A is an essential nutrient acquired from the diet as carotenoids in fruit and vegetables, and as retinyl esters in foods of animal origin. The absorption of dietary vitamin A involves the hydrolyzation of dietary retinyl esters in the intestine, re-esterification in enterocytes and incorporation to chylomicrons containing dietary lipids (Harrison 2001, Mutanen 1993; Figure 2). The chylomicrons enter the blood stream through the thoracic duct. The chylomicron remnants containing the retinyl esters are taken up by the hepatocytes. Besides the chylomicron route, some of the free, unesterified retinol is transported directly to the portal circulation (Nayak 2001).

Vitamin A is stored in the liver and in adipose tissue, and the plasma vitamin A levels are kept constant through homeostatic regulation from the liver stores (Biesalski 2004). Before being released to the blood stream, the intrahepatic retinol is bound to the retinol binding protein.

*Figure 2. Absorption and metabolism of vitamin A. RBP, retinol-binding protein. After Harrison 2001 and Mutanen 1993.*
Role of vitamin A in gut immunity

To become activated, a naïve T cell must be introduced to a foreign antigen by an antigen-presenting cell, such as the dendritic cell (Steinman 2005). Thereafter, the activated T cell migrates and homes to some specific compartment of the body, such as the skin or the intestine, guided by integrins and chemokine receptors expressed on the cell surface. Vitamin A induces the expression of the gut-homing chemokine receptor CCR9 and the integrin α4β7 on T cells (Iwata 2004). The dendritic cells isolated from the gut-associated lymphoid tissue are able to convert vitamin A to its active form, retinoic acid, which induces the expression of CCR9 and α4β7 on the T cells (Figure 3).

Removing vitamin A from the diet was shown to result in a dramatic depletion of CD4-positive T cells from the intestinal mucosa but not from e.g. the lung in mice (Iwata 2004). In addition, the retinoid acid produced by dendritic cells induces the gut-homing molecules CCR9 and α4β7 on activated B cells, and, in the presence of IL-5 or IL-6, the production of IgA (Mora 2006). Furthermore, the dendritic cells of the gut-associated lymphoid tissue have been shown to convert T cells into regulatory Foxp3 positive cells involved in oral tolerance. This TGF-β-dependent conversion is enhanced by retinoic acid, which may also suppress the generation of the inflammatory T helper 17 cells (von Boehmer 2007, Benson 2007, Kang 2007).
Figure 3. Retinoic acid generated by the dendritic cells of the gut-associated lymphatic tissue (GALT DC) imprint the gut-homing specificity on T cells by inducing the gut-homing molecules CCR9 and α4β7 on T cells and downregulating the skin-homing molecules E-selectin ligand and CCR4. After Johansson-Lindbom 2004.

Association of vitamin A and atopic manifestations

Children with asthma may have lower concentrations of serum vitamin A, α- and β-carotene than healthy controls (Arora 2002, Harik-Khan 2004, Rubin 2004). In children with a family history of allergy, a low intake of retinol in infancy has been associated with childhood atopic eczema (Laitinen 2005).
Retinoic acid, the active metabolite of vitamin A, has been shown to modulate the differentiation of the hematopoietic cells by promoting neutrophil differentiation and by suppressing eosinophil differentiation (Paul 1995, Denburg 2001, Kinoshita 2000), to inhibit the growth and activation of mast cells (Ishida 2003), and to inhibit the production of eotaxin, an eosinophil chemoattractant (Takamura 2003). Atopic subjects have higher numbers of circulating eosinophil/basophil colony-forming units than nonatopics (Denburg 1985), and blood eosinophilia during infancy is associated with subsequent allergic disease in childhood (Borres 2004).

Retinoic acid was shown to inhibit the IL-4-mediated proliferation and IgE production of B cells in vitro (Worm 1998). Retinoic acid inhibited IgE production more effectively in B cells from atopic dermatitis patients with low total IgE levels than in B cells from patients with high total IgE, whereas in an in vivo model using mice sensitized with ovalbumin, the retinoids did not inhibit IgE production (Worm 2001). Vitamin A derivatives increase the prostaglandin biosynthesis in vivo (Devaux 2001) and regulate the expression and activity of several enzymes involved in the host defense against pathogens (Grosjean 2001, Hill 1996). Recently, retinoic acid was shown to be a regulator of TGF-β-dependent immune responses, capable of inhibiting the induction of proinflammatory T helper 17 cells and promoting the differentiation of anti-inflammatory T regulatory cells (Mucida 2007).

**Lipid metabolism and atopy**

**Cholesterol and serum lipoproteins**

**Cholesterol**

Serum cholesterol is mainly derived from the liver, where it is synthesized from acetyl coenzyme A in the endoplasmic reticulum of the hepatocytes (Goldstein 1990). Liver also converts cholesterol into bile acids, which form the major route of excretion from the body (Dietschy 1970). As well as being a precursor of the synthesis of sterol hormones, cholesterol is an important structural component of the cell membranes. It modulates the fluidity of the membranes and, together with sphingomyelin, forms lipid rafts, specialized plasma membrane domains rich in cholesterol and with a signal transduction potential (Brown 1999, Simons 2000). The lipid rafts have been implicated in regulating e.g. the mast cell activation (Field 1997, Sheets 1999). In vitro, cholesterol depletion has been shown to enhance high affinity IgE receptor-mediated mast cell degranulation and cytokine production (Kovarova 2006, Surviladze 2001).
**Triglycerides**

Triglycerides consist of three fatty acid molecules attached by an ester linkage to a glycerol molecule. They represent the storage or carrier form of fatty acids in plasma and tissue, and are formed by hepatocytes and adipose cells (Kovanen 2000). In addition to the fatty acids in the triglycerides, a fraction of fatty acids are transported in the blood as free fatty acids bound to albumin.

**Serum lipoproteins**

Being water-insoluble lipids, cholesterol and triglycerides are carried in the blood by lipoproteins. The lipoproteins are spherical particles composed of cholesteryl esters and triglycerides surrounded and solubilized by a surface monolayer of phospholipid and unesterified cholesterol, and stabilized by an apolipoprotein. The serum lipoproteins comprise a continuum of particles with varying densities, lipid and apolipoprotein composition; however, several major classes have been defined. These are the chylomicrons of dietary origin, the very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), the low-density lipoproteins (LDL), and the high-density lipoproteins (HDL) (Kovanen 2000).

**Apolipoproteins**

The apolipoproteins provide structural stability for the lipoproteins and regulate the lipoprotein metabolism. Ten major and six minor apolipoproteins have been defined. Of the major apolipoproteins, apolipoprotein B 100 (ApoB-100) is the major apolipoprotein of VLDL, IDL, and LDL and acts as the ligand for receptor-mediated uptake of LDL. Polymorphism of ApoB-100 partly accounts for the genetic variation in LDL cholesterol levels (Aalto-Setälä 1989). Apolipoprotein E (ApoE) has a crucial role in lipoprotein metabolism as a ligand for receptors of the LDL receptor superfamily (Mahley 1999). The ApoE gene exhibits polymorphism with three alleles e2, e3, and e4 (Utermann 1977). Of these, the e4 allele is associated with increased serum LDL and triglyceride levels as well as with an increased risk of coronary heart disease (Tammi 2000, Stengard 1998, Kuusi 1989).
Dietary polyunsaturated fatty acids and T cell immunity

Parallel to the increase of allergic diseases, the consumption of saturated fat and n-3 polyunsaturated fatty acids has decreased and that of n-6 polyunsaturated fatty acids has increased in industrialized countries, and these alterations in dietary fat intake are considered as possible contributing causes of the increase in allergic diseases (Black 1997, Porkka 1997, Sanders 2000).

The n-6 and n-3 polyunsaturated fatty acids are precursors of eicosanoids (Calder 1998; Figure 4). Linoleic acid (18:2 n-6), the main n-6 polyunsaturated fatty acid, is converted to arachidonic acid (20:4 n-6), a precursor of the proinflammatory two-series prostaglandins and the four-series leukotrienes (e.g. prostaglandin E2 and leukotriene B4). Eicosanoids derived from arachidonic acid are considered important mediators of the allergic inflammation. On the other hand, the main n-3 polyunsaturated fatty acid, α-linolenic acid (18:3 n-3) is metabolized to eicosapentaenoic acid (20:5 n-3), which is a precursor of the less inflammatory three-series prostaglandins and five-series leukotrienes, and which competitively inhibits the metabolism of arachidonic acid.

Prostaglandin E2 inhibits the production of T helper type 1 cytokines, but does not affect the production of T helper type 2 cytokines, and stimulates the IgE-production by B lymphocytes (Calder 2002). Therefore, the increased dietary n-6/n-3 fatty acid ratio is thought to favour the development of a T helper type 2-related inflammatory response by providing an abundance of prostaglandin E2 precursors as well as reduced inhibition of prostaglandin E2 synthesis. However, the interaction between the n-6 and n-3 polyunsaturated fatty acids and the immune system as well as the role of prostaglandin E2 may be more complex than previously supposed. According to some studies, prostaglandin E2 may protect against airway inflammation and bronchoconstriction, and in an animal model, the net effect of prostaglandin E2 appears to be one of inhibition of T helper 2 responses (Pavord 1995, Gauvreau 1999, Martin 2002). Furthermore, there is evidence that the polyunsaturated fatty acids may inhibit cytokine production in a prostaglandin E-independent mechanism (Santoli 1990, Santoli 1989).
Epidemiological associations of polyunsaturated fatty acid intake and allergic diseases

Several studies demonstrate an altered dietary and serum polyunsaturated fatty acid composition with higher levels of $n$-6 or lower levels of $n$-3 polyunsaturated fatty acids in atopic subjects (Dunder 2001, Leichsenring 1995, Wakai 2001, Solvoll 2000), though studies with conflicting results exist (Bolte 2006, Troisi 1995). Consumption of fish or fish oil rich in $n$-3 polyunsaturated fatty acids has been associated with a decreased risk of childhood asthma (Hodge 1996, Nafstad 2003). However, in
randomized controlled trials, \( n-3 \) polyunsaturated fatty acid supplementation has not been shown to have a beneficial effect on established asthma in adults or in children (Woods 2002). Furthermore, dietary \( n-3 \) polyunsaturated fatty acid supplementation and restriction of dietary \( n-6 \) fatty acids failed to prevent the development of asthma, eczema or sensitization by the age of 5 years in a randomized trial on infants with a family history of asthma (Almqvist 2007).

Maternal diet rich in \( n-6 \) polyunsaturated fatty acids during the last month of pregnancy has been suggested to be associated to an increased risk of allergic diseases in the offspring at 2 years of age, whereas a high intake of \( n-3 \) polyunsaturated fatty acids had a protective effect (Sausenthaler 2007). Fish oil supplementation during pregnancy has been shown to result in higher proportion of \( n-3 \) polyunsaturated fatty acids in neonatal erythrocyte membranes and in lower neonatal cytokine responses to allergens as well as in higher levels of \( n-3 \) polyunsaturated fatty acid, immunoglobulin A and soluble CD14 bacterial pattern recognition receptor in the breast milk. Furthermore, the fish oil supplementation during pregnancy was associated with a decreased risk of skin prick test reactivity and a milder degree of atopic dermatitis at age 1 year (Dunstan 2003, Dunstan 2004). Contrary to these results, the fetal exposure to a high ratio of \( n-6 \) to \( n-3 \) polyunsaturated fatty acids, as measured by erythrocyte fatty acid composition in cord blood and in maternal blood in late pregnancy, was not a significant risk factor of childhood wheezing and eczema (Newson 2004).

Breast milk fatty acid composition may be associated with the development of atopic symptoms in the infant (Duchén 1998). In a Swedish study, breast milk from allergic mothers was shown to contain low levels of \( n-3 \) and \( n-6 \) polyunsaturated fatty acids, and a high arachidonic acid \((n-6)\): eicosapentaenoic acid \((n-3)\) ratio in breast milk was related to the development of allergic symptoms in the infant (Duchén 2000). According to a Dutch study, the breast milk \( n-3 \) polyunsaturated fatty acids and the ratio between the \( n-3 \) and the \( n-6 \) polyunsaturated fatty acids were inversely associated with the occurrence of asthma and eczema in children of allergic mothers (Wijga 2006). Conflicting results were reported from an Australian study, in which breast milk fatty acid profile was found to be independent of maternal atopy and high levels of \( n-3 \) fatty acids in breast milk were associated with increased risk of atopy in the infant (Stoney 2004).

### Dietary polyunsaturated fatty acids and serum cholesterol

A high intake of saturated fatty acids increases serum cholesterol (Grundy 1988), whereas linoleic acid, the major dietary \( n-6 \) polyunsaturated fatty acid, has an opposite effect (Mensink 1987). Therefore, if the increased intake of \( n-6 \) fatty acids is a causative factor for atopic diseases, total cholesterol may be expected to be inversely associated with manifestations of atopy (Schäfer 2003).

Data from cross-sectional studies indicate that a low level of serum cholesterol is associated with the occurrence of atopic diseases (Joki 2003, Shenoi 1992, Schäfer
Joki et al. found that young children with food allergy have lower total cholesterol than healthy children, and 26-45% of the children with food allergy also had a low HDL cholesterol concentration. The authors speculated that the restricted diet of the children with food allergy contributed to the low cholesterol concentrations. Shenoi et al. reported significantly lower total and HDL cholesterol levels in children with respiratory allergy, i.e. asthma or allergic rhinitis than in the nonatopic controls. Schäfer et al. studied adults aged 27-78 years in a case-control study and found a positive association between the HDL cholesterol level and the frequency of allergic rhinoconjunctivitis and atopic eczema. In addition, they found a negative association between total and LDL cholesterol levels and the frequency of allergic rhinoconjunctivitis and allergic sensitization, but these were no longer significant after adjustment for covariates.
AIMS OF THE STUDY

1. To clarify whether the allergy preventive effect of breastfeeding can be enhanced by prolonging the period of strictly exclusive breastfeeding above the generally recommended 6 months of age.

2. To find out which is the allergy predictive capacity of cord serum IgE in a cohort of unselected healthy infants with a follow-up period extended to adulthood.

3. To find out whether the plasma levels of retinol, crucial in imprinting the gut-homing property to T-cells, in infancy and childhood are associated with subsequent development of atopic manifestations.

4. To determine whether the serum total, LDL and HDL cholesterol levels in infancy and childhood are associated with subsequent development of atopic manifestations.
SUBJECTS AND METHODS

Study population

The present studies comprise a part of a 20-year prospective follow-up study on 200 healthy Finnish newborns. The study was initiated in 1981 in order to study the nutritional effects of exclusive breastfeeding (Kallio 1992, Salmenperä 1985). The participating newborns and their families were recruited during their stay at the maternity hospital. All the participants were enrolled between June and September in 1981. The inclusion criteria were a healthy full-term infant with appropriate weight for gestational age, a 1-min Apgar score of at least 8, and a healthy, nonsmoking mother with uncomplicated pregnancy and delivery.

Of the families invited, 90% agreed to participate the study. Since only two families dropped out during the first year, 198 infants, 114 girls and 84 boys, completed the first-year follow-up (Kallio 1992, Salmenperä 1985).

Infant feeding

Exclusive breastfeeding was defined as the infant being fed exclusively maternal human milk without any supplementary formula or solid foods, only water and routine vitamins were allowed. All the mothers begun and were encouraged to maintain strictly exclusive breastfeeding for as long as possible. Thus, all the infants received some length of exclusive breastfeeding. If supplementary milk was needed, the infants were given donated human milk at the maternity hospital and at home. Exclusive breastfeeding was continued until it became insufficient for the child. This was verified by offering the child a dose of donated human milk after breastfeeding. When supplemental human milk had been needed twice, exclusive breastfeeding was considered terminated, and the child was gradually weaned to a cow’s-milk-based formula (Tutteli®; Valio, Helsinki, Finland). For the infants no longer on exclusive breastfeeding, solid foods were introduced from age 3 months, while for those still on exclusive breastfeeding, the introduction of solid foods was postponed until the exclusive breastfeeding was discontinued. In accordance to a national recommendation, the infants received vitamin A (1000 IU/day) and vitamin D (400 IU/day) supplementation (Vitol®, Orion, Finland) from birth through the second year of life.

The mothers and their infants were seen at the outpatient clinic of the Hospital for Children and Adolescents routinely at ages 2, 4, 6, 9 and 12 months and those still on exclusive breastfeeding were also seen at ages 10 and 11 months. In addition, all medical care and counseling needed during the infant’s first year were provided by the pediatrician responsible for the first-year follow-up. The families were free to contact
the pediatrician and to visit the outpatient clinic between the scheduled visits, whenever necessary. Due to the careful follow-up and frequent contacts between the visits, the exact duration of exclusive breastfeeding was reliably recorded.

**Duration of exclusive breastfeeding**

For the analytical purposes of this study, the subjects were divided into four groups according to the duration of exclusive breastfeeding they had received, i.e. exclusive breastfeeding for < 2 months, 2 - < 6 months, 6 - < 9 months and ≥ 9 months. The proportion of the infants on exclusive breastfeeding was 82% at age 2 months, 58% at age 6 months, and 18% at age 9 months. Children with a family history of allergy and those without a family history of allergy were distributed similarly among the four study groups (Table 1).

*Table 1. Infants with a family history of allergy (FHA) and those without a family history of allergy were distributed similarly among the four study groups (study I, Pesonen 2006).*

<table>
<thead>
<tr>
<th>Duration of exclusive breastfeeding</th>
<th>Total</th>
<th>Negative FHA</th>
<th>Positive FHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 months</td>
<td>35 (18%)</td>
<td>19 (17%)</td>
<td>16 (19%)</td>
</tr>
<tr>
<td>2 - &lt; 6 months</td>
<td>48 (24%)</td>
<td>27 (24%)</td>
<td>21 (25%)</td>
</tr>
<tr>
<td>6 - &lt; 9 months</td>
<td>80 (40%)</td>
<td>49 (43%)</td>
<td>31 (37%)</td>
</tr>
<tr>
<td>≥ 9 months</td>
<td>35 (18%)</td>
<td>19 (17%)</td>
<td>16 (19%)</td>
</tr>
<tr>
<td>N</td>
<td>198</td>
<td>114</td>
<td>84</td>
</tr>
</tbody>
</table>

**Family history of allergy**

Family history of allergy was considered positive if the child had a first-degree relative, i.e. mother, father or sibling, with allergic symptoms during the infant’s first year of life. Of the 198 children, 84 (42%) had a family history of allergy. Fifty-two (26%) children had a maternal, 33 (17%) a paternal, and 7 (4%) a biparental family history of allergy.
Follow-up examinations

Of the 198 children, 163 (82%) were re-assessed at age 5 years, 150 (76%) at age 11 years, and 164 (83%) at age 20 years with clinical examination, SPT, and parental (ages 5 and 11 years) and personal (age 20 years) structured interviews by a single physician (Markku J.T. Kallio at the 5- and 11-year re-examinations, Maria Pesonen at the 20-year re-examination) unaware of the early feeding history. Prior to the 5- and 11-year visits, the parents filled in a questionnaire concerning the possible allergic symptoms in the child, and the information in the questionnaires was confirmed by personal interviews.

Definitions of allergic symptoms

Atopic dermatitis was defined as a chronic or relapsing itchy dermatitis with a characteristic clinical morphology and distribution (Hanifin 1980). Atopic dermatitis was recorded if it was present at a follow-up examination, or atopic dermatitis had been diagnosed by a physician and there was a history of relapsing eczema with typical localization during the preceding year.

Allergic rhinitis was defined as recurrent non-infectious rhinitis at pollen seasons or on aeroallergen exposure. Allergic conjunctivitis was defined as itching and watery discharge of the eyes at pollen seasons or on aeroallergen exposure.

Recurrent wheezing was recorded if the subject had a diagnosis of bronchial asthma, or two or more separate episodes of respiratory distress and wheezing.

Food hypersensitivity symptoms were defined as a history of repeated itching or swelling of the lips, the oral mucosa or the throat, urticarial eruption, or severe vomiting after ingestion of a specific food. Since performing double-blind placebo-controlled food challenges (Young 1994) was not feasible, we chose to rely on the history of food hypersensitivity, which was recorded in a personal interview by a physician and based on reports of typical symptoms appearing after ingestion of a specific food. At age 20 years, food hypersensitivity symptoms were no longer taken into account.

Verified atopy was recorded if the subject had one or several allergic symptoms and a positive SPT to at least one of the common allergens tested.

Skin prick testing (SPT)

SPTs were performed on 160 at age 5 years, on 149 at age 11 years, and on 164 subjects at age 20 years with standardized allergen extracts (ALK, Copenhagen, Denmark) of birch, alder, timothy, Kentucky bluegrass, mugwort, cat, dog, Dermatophagoides pteronyssinus, cow’s milk, egg and codfish; histamine hydrochloride solution (10 mg/ml) and a diluent control were included. A positive
SPT was defined as a test reaction with a mean diameter equal to or greater than half of the diameter of the histamine wheal (Meinert 1994).

**Determination of serum immunoglobulin E (IgE)**

Cord blood serum IgE (CS-IgE) was determined in 190 of the 200 newborns from undiluted samples by a paper radioimmunoabsorbent test (Phadebas IgE PRIST®, Pharmacia, Uppsala, Sweden). Based on the lower analytical limit of the method, the CS-IgE values were dichotomized into < 0.5 kU/L and ≥ 0.5 kU/L (Sadeghnejad 2004, Tariq 1999).

Total serum IgE at ages 5 (n = 161) and 20 years (n = 162) was determined by the Pharmacia ImmunoCAP System IgE FEIA® (Pharmacia Diagnostics, Uppsala, Sweden). The samples were stored at -20 °C until analyzed in 2004. The samples at age 11 years (n = 126) were analyzed by Pharmacia CAP System IgE FEIA® in 1993. The cut-off for an elevated total IgE was set to 130 kU/L at ages of 11 and 20 years, and to 48 kU/L at the age of 5 years (Johansson 1988).

**Determination of plasma concentration of vitamin A**

The retinol concentration was repeatedly measured in infancy in every other infant entering the study, and in childhood (at 5 and 11 years of age) in all of the participants of the re-examinations. Therefore, the plasma concentration of retinol was determined in cord blood at birth (n = 97), in plasma samples at ages 2, 4 and 12 months (n = 95), and at ages 5 (n = 155) and 11 years (n = 151). Blood plasma was separated and frozen within 30 minutes, stored at -18 °C, protected from light exposure, and transported in dry ice by air to the Department of Human Nutrition and Health, F. Hoffmann-La Roche & Co. Ltd. Switzerland, where the vitamin A concentration in plasma was determined by high-performance liquid chromatography (Vuilleumier 1983).

**Determination of serum total cholesterol, high-density and low-density lipoprotein cholesterol**

Serum total cholesterol was determined in cord blood at birth (n = 193), at ages of 2 (n = 192), 4 (n = 192), 6 (n = 190), 9 (n = 188), and 12 months (n = 196), and at ages of 5 (n = 160), 11 (n = 150) and 20 years (n = 162). Low-density (LDL) and high-density (HDL) lipoprotein cholesterol concentrations were determined at ages of 5, 11 and 20 years.

After centrifugation of the blood, the cells and the serum were separated, and the serum samples were stored at +4 °C until analyzed. All the cholesterol analyses were performed within 24h of blood sampling. Serum total cholesterol was determined
with an AutoAnalyzer (Huang 1961). Lipoprotein cholesterol concentration was measured by an enzymatic method (Röschlau 1974). Cholesterol was quantified from the infranatant, and HDL cholesterol was determined after heparin-manganese precipitation of apo B-containing particles (Bethesda 1974). The difference between the total cholesterol and HDL cholesterol values in the infranatant gives the LDL cholesterol value.

**Statistical methods**

Statistical analyses were performed with StatView 5.0 (SAS Institute; Cary, NC). Chi-square, Fisher’s exact and Student’s t tests and analysis of variance for repeated measurements were used to measure statistical significance, and \( p < 0.05 \) was taken as indicating statistical significance. Cholesterol and retinol concentrations were log transformed before analyses. Multivariate logistic regression was used for examining the effect of confounding factors. The potential confounding factors taken into account were maternal age (< 26 or ≥ 26 years), maternal educational level (high or intermediate/low), family history of allergy, infant’s gender, smoking in the household during the infant’s first year, sibship size (< 3 or ≥ 3), daycare attendance at age 1 year, and personal smoking at age 20 years. When assessing the effect of the duration of exclusive breastfeeding on subsequent atopic symptoms, the multivariate logistic regression analyses were calculated separately for subjects with and without a family history of allergy.

For evaluating the usefulness of cord serum IgE as a predictor of subsequent atopic manifestations, sensitivity (SE), specificity (SP) and positive predictive values (PPV) were calculated. SE is defined as the probability that the test is positive given that the patient has the disease, and calculated as \( \frac{\text{true positives}}{\text{true positives} + \text{false negatives}} \). SP is defined as the probability that the test is negative given that the patient does not have the disease, and it is calculated as \( \frac{\text{true negatives}}{\text{true negatives} + \text{false positives}} \). PPV is the proportion of true positives out of all positive results, i.e. it is the probability that a patient with a positive test result has the disease, and it is calculated as \( \frac{\text{true positives}}{\text{true positives} + \text{false positives}} \) (Hansen 1992a). Positive likelihood ratio (LR+) is calculated as \( \frac{\text{SE}}{1 - \text{SP}} \). In contrast with PPV, it has the advantage of not being dependent of the prevalence of the disease in the study population.

**Ethical considerations**

The study was approved by the Ethical Review Board of the Hospital for Children and Adolescents and the Ethical Review Board for Internal Medicine, Helsinki University Hospital. Written informed consent was obtained from the parents at the beginning of the follow-up study, and from the participants of the 20-year re-examination.
RESULTS

Frequency of atopic manifestations in infancy, childhood and adolescence

As previously published, 14 (7%) children showed signs of atopy during their first year of life (Savilahti 1987). At age 5 years, one or several allergic symptoms were recorded in 40 (25%) of the 160 children seen at that age. The prevalence of allergic symptoms then increased to 46% at age 11 years and to 52% at age 20 years. Throughout the 20-year follow-up, the boys had a higher frequency of allergic symptoms than the girls, though the differences were not statistically significant.

At age 5 years, the most frequent symptoms were atopic dermatitis (18%) and food hypersensitivity (12%). Of the 28 children with atopic dermatitis, 11 (39%) had symptoms of food hypersensitivity as well. Low prevalences of allergic rhinitis (3%) and allergic conjunctivitis (4%) were recorded at age 5 years, but they increased to 23% and 20% at age 11 years, and to 41% and 31% at age 20 years, respectively (Figure 5). The prevalence of food hypersensitivity symptoms was at the same level at ages 5 and 11 years, 12% and 11%, respectively.

Of the 28 children with atopic dermatitis at age 5 years, 21 (75%) and, of the 19 children with food hypersensitivity symptoms, 12 (63%) were skin prick test (SPT) negative, whereas SPT positivity was observed in 10% of the 120 symptom-free children. Of the 16 children with food hypersensitivity symptoms at 11 years of age, 8 (50%) were SPT negative, whereas 20% of the 81 symptom-free children were SPT positive. At age 20 years, 67 (79%) of the 85 subjects with allergic symptoms and 24% of the 79 symptom-free subjects were SPT positive.
Effect of family history of allergy on the prevalence of allergic symptoms

At ages 5 and 20 years, higher prevalences of allergic symptoms (p = 0.01 and 0.04), SPT positivity (p = 0.04 and 0.03) and verified atopy (p = 0.03 and < 0.01) were observed in the children with a family history of allergy than in those without a family history of allergy. At age 11 years, the prevalence of allergic symptoms was similar, whereas the prevalence of SPT positivity was higher in the children with a family history of allergy than in those without a family history of allergy (p = 0.02).

The effect of maternal heredity on the prevalence of allergic symptoms appeared stronger than that of paternal heredity, the prevalences being 41%, 50% and 72% at ages 5, 11 and 20 years among the subjects with a maternal heredity, 22%, 35% and 55% among the subjects with a paternal heredity, and 71%, 80% and 100% among the 7 subjects with a biparental history of atopy, respectively.

Figure 5. Prevalence (%) of allergic symptoms, i.e. atopic dermatitis, allergic rhinitis, allergic conjunctivitis, recurrent wheezing, food hypersensitivity symptoms, at ages of 5, 11 and 20 years.
Demographic characteristics of the study population

Smoking in the household during the infant’s first year occurred in 34 families, and it was recorded more often in the group with exclusive breastfeeding for < 2 months (28%, p = 0.05) than in the infants with exclusive breastfeeding for 2 - < 6 months, 6 - < 9 months or ≥ 9 months. Day care attendance at age 1 year was least frequent, 57%, in the group with exclusive breastfeeding for < 2 months and most frequent, 85% (p = 0.04), in the group with exclusive breastfeeding for 2 - < 6 months.

Effect of prolonged exclusive breastfeeding on subsequent allergic symptoms

Exclusive breastfeeding prolonged for ≥ 9 months was associated with atopic dermatitis (p = 0.002) and symptoms of food hypersensitivity (p = 0.02) at age 5 years, and with symptoms of food hypersensitivity at age 11 years (p = 0.01), in children with a family history of allergy (I).

Among the 72 children with a family history of allergy seen at age 5 years, the prevalence of allergic symptoms was highest, 56%, in the children who had exclusive breastfeeding for ≥ 9 months (OR 3.3, CI 1.0-10.3, p = 0.01), whereas the prevalence was 36%, 20% and 30% in children with exclusive breastfeeding for < 2 months, for 2 - < 6 months, and for 6 - < 9 months. Atopic dermatitis was recorded in 17 (24%) and symptoms of food hypersensitivity in 12 (17%) of the 72 children with a family history of allergy. The highest prevalences of atopic dermatitis, 44% (OR 3.7, 95% CI 1.1-12.1, p = 0.03; Figure 6), and food hypersensitivity symptoms, 38% (OR 5.1, 95% CI 1.3-19.1, p = 0.01), were recorded in the children who had received exclusive breastfeeding for 9 months or more.
At 11 years of age, allergic symptoms were recorded in 30 (46%) of the 65 children with a family history of allergy. Among these, food hypersensitivity symptoms were recorded most often in the children who had received exclusive breastfeeding for ≥ 9 months (43%; OR 6.9, 95% CI 1.7-28.1, p < 0.01). None of the children with a family history of allergy and exclusive breastfeeding for 2 - < 6 months had atopic dermatitis at age 11 years (Figure 6). The association of prolonged exclusive breastfeeding and subsequent allergic symptoms at ages 5 and 11 years remained significant after adjusting for potential confounding factors (Table 2).

Among the 88 children without a family history of allergy seen at age 5 years, the prevalence of atopic dermatitis was highest, 28% (OR 4.2, 95% CI 1.1-15.9, p = 0.02), in those who had received exclusive breastfeeding for ≥ 9 months, the prevalence being 0, 11%, and 11% in children with exclusive breastfeeding for < 2 months, for 2 - < 6 months, and for 6 - < 9 months (Figure 6). However, when the potential confounders were taken into account, the difference was not significant (Table 2). Thus, exclusive breastfeeding for ≥ 9 months was not significantly associated with allergic symptoms or SPT positivity among the subjects without a family history of allergy.

Figure 6. Influence of the duration of exclusive breastfeeding on the prevalence (%) of atopic dermatitis at the ages of 5, 11, and 20 years. FHA+, subjects with a family history of allergy; FHA-, subjects without a family history of allergy (Study I, Pesonen 2006).
Table 2. The effect of exclusive breastfeeding prolonged for over 9 months as compared to a shorter duration of exclusive breastfeeding in children with a family history of allergy (positive FHA) and in children without a family history of allergy (negative FHA) calculated with multivariate logistic regression* (Study I, Pesonen 2006).

<table>
<thead>
<tr>
<th>Positive FHA</th>
<th>Negative FHA</th>
<th>Total population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OR (95%CI)</strong></td>
<td><strong>P</strong></td>
<td><strong>OR (95%CI)</strong></td>
</tr>
<tr>
<td><strong>5 years of age (n=132)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic symptoms</td>
<td>6.1 (1.5-24.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>6.5 (1.5-28.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>Food hypersensitivity</td>
<td>5.3 (1.2-24.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>Verified atopy†</td>
<td>0.1 (0.2-6.4)</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>11 years of age (n=126)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic symptoms</td>
<td>0.7 (0.2-2.6)</td>
<td>0.61</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>0.2 (0.0-2.1)</td>
<td>0.19</td>
</tr>
<tr>
<td>Food hypersensitivity</td>
<td>7.9 (1.4-50.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Verified atopy†</td>
<td>0.4 (0.1-1.5)</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>20 years of age (n=132)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic symptoms</td>
<td>1.9 (0.4-8.7)</td>
<td>0.43</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>2.7 (0.3-21.6)</td>
<td>0.36</td>
</tr>
<tr>
<td>Verified atopy†</td>
<td>1.5 (0.3-6.6)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

* Independent variables used in the model: Duration of exclusive breastfeeding (< 9 months / ≥ 9 months), gender, maternal age, maternal educational level, smoking in the household during the first year, daycare attendance at the age of 1 year, sibship size 3 or more.
† Allergic symptoms and SPT positivity
Prediction of subsequent atopic manifestations by cord serum IgE and family history of allergy

Cord serum IgE (CS-IgE) was successfully measured in 190 of the 200 newborns enrolled in the follow-up study (IV). An elevated CS-IgE (≥ 0.5 kU/L) was recorded in 38 infants (20%). The values were within the range of 0.5 – 9.1 kU/L in 37 newborns. Because an exceptionally high CS-IgE value might be due to contamination with maternal blood, we excluded from further analyses one newborn with CS-IgE value of 44.4 kU/L. The occurrence of an elevated CS-IgE was similar in boys and in girls, and in those with and without a history of smoking in the household. Of the 79 infants with a family history of allergy, 23% had an elevated CS-IgE, but also 17% of the 110 infants without a family history of allergy had an elevated CS-IgE.

CS-IgE value and atopic manifestations at age 5 years

An elevated CS-IgE was associated with subsequent allergic symptoms and SPT positivity at age 5 years (Figure 7). Of the children with an elevated CS-IgE, 33% had atopic dermatitis at age 5 years, whereas it was recorded in 15% of the children without an elevated CS-IgE (p = 0.02). Food hypersensitivity symptoms were more frequent among the children with an elevated CS-IgE (28%) than in those without (10%, p = 0.02). In addition, SPT positivity (n = 24) and verified atopy (n = 12) at age 5 years were associated with an elevated CS-IgE (p = 0.02 and 0.01, respectively).
**Figure 7.** Prevalence of allergic symptoms, skin prick test (SPT) positivity and verified atopy (i.e. allergic symptoms and SPT positivity) at age 5 years in children with and without an elevated CS-IgE (Study II, Pesonen 2008). AD, atopic dermatitis; FHS, food hypersensitivity symptoms; ARC, allergic rhinoconjunctivitis; RW, recurrent wheezing; SPT, skin prick test positivity; VA, verified atopy. Significant differences marked with asterisks (*p < 0.02, **p < 0.01).

**CS-IgE value and atopic manifestations at ages of 11 and 20 years**

We observed an association between an elevated CS-IgE and the occurrence of allergic rhinoconjunctivitis at ages of 11 and 20 years (p = 0.04 and 0.02, respectively; (Table 3). Of the children with an elevated CS-IgE, 41% had allergic rhinoconjunctivitis at age 11 years, whereas it was recorded in 25% of those without an elevated CS-IgE. The corresponding percentages were 58% and 37% at age 20 years. Moreover, an elevated CS-IgE was associated with an elevated serum total IgE at ages of 11 and 20 years (p = 0.02 and 0.01, respectively).
Table 3. Association of an elevated CS-IgE and atopic manifestations at ages of 5, 11 and 20 years as calculated with logistic regression* (Study II, Pesonen 2008).

<table>
<thead>
<tr>
<th>Outcomes at age 5 years</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic dermatitis (n=26)</td>
<td>3.9 (1.3-11.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Food hypersensitivity symptoms (n=18)</td>
<td>4.3 (1.2-14.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>Any allergic symptom (n=37)</td>
<td>2.8 (1.1-7.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>SPT† positivity (n=24)</td>
<td>4.6 (1.7-12.7)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcomes at age 11 years</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food hypersensitivity symptoms (n=16)</td>
<td>3.9 (0.9-16.9)</td>
<td>0.06</td>
</tr>
<tr>
<td>Allergic rhinoconjunctivitis (n=40)</td>
<td>2.6 (1.0-6.9)</td>
<td>0.05</td>
</tr>
<tr>
<td>Elevated total IgE (≥130 kU/L) (n=27)</td>
<td>3.2 (1.2-8.7)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcomes at age 20 years</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic rhinoconjunctivitis (n=65)</td>
<td>2.7 (1.1-6.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Any allergic symptom (n=81)</td>
<td>2.4 (1.0-5.7)</td>
<td>0.04</td>
</tr>
<tr>
<td>Elevated total IgE (≥130 kU/L) (n=41)</td>
<td>3.7 (1.6-8.8)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Independent variables used in the model: CS-IgE (≥ 0.5kU/L as the reference value), gender, maternal atopy, sibship size, smoking in the household during the infant’s first year, duration of exclusive breastfeeding. Smoking status at age 20 years was added for the outcomes at age 20 years.
† Skin prick test

Since a prolonged exclusive breastfeeding was associated with an increased prevalence of atopic dermatitis at age 5 years, and food hypersensitivity symptoms at ages of 5 and 11 years, exclusive breastfeeding was considered a potential confounder and included as a cofactor in the logistic regression analyses. However, the CS-IgE level was not associated with the duration of exclusive breastfeeding, i.e. children with an elevated CS-IgE and those with CS-IgE < 0.5 kU/L were equally likely to receive a prolonged exclusive breastfeeding (Fisher’s exact p = 0.8).

CS-IgE combined with family history of allergy as a predictor of subsequent atopic manifestations

A combination of elevated CS-IgE and positive family history of allergy predicted subsequent atopic manifestations more accurately than CS-IgE or family history of allergy alone, as is indicated by the higher likelihood ratios (Table 4). The
combination of elevated CS-IgE and positive family history of allergy was associated with SPT positivity, allergic symptoms and verified atopy at age 20 years (Figure 8) as well as with SPT positivity at age 11 years (p = 0.05) and SPT positivity, allergic symptoms and verified atopy at age 5 years (p < 0.001, 0.01 and 0.001, respectively). Whereas the specificities and positive predictive values were increased, the sensitivities of the combination of CS-IgE and family history of allergy were decreased as compared to those of CS-IgE or family history of allergy (Table 4).

**Table 4.** Comparison of the predictive value of CS-IgE ≥ 0.5kU/L (CS-IgE+), positive family history of allergy (FHA+), and a combination of CS-IgE ≥ 0.5kU/L and a positive family history of allergy (CS-IgE+ and FHA+) on atopic manifestations at ages of 5 and 20 years (Study II, Pesonen 2008). Sensitivity (SE), specificity (SP) and positive predictive value (PPV) given as percentages. LR+, positive likelihood ratio.

<table>
<thead>
<tr>
<th>Outcomes at 5 years</th>
<th>CS-IgE+</th>
<th>FHA+</th>
<th>CS-IgE+ and FHA+</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE</td>
<td>SP</td>
<td>PPV</td>
<td>LR+ SE</td>
</tr>
<tr>
<td>Allergic symptoms</td>
<td>30</td>
<td>85</td>
<td>38 2.0</td>
</tr>
<tr>
<td>SPT† positivity</td>
<td>38</td>
<td>84</td>
<td>30 2.4</td>
</tr>
<tr>
<td>Verified atopy‡</td>
<td>50</td>
<td>83</td>
<td>20 2.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcomes at 20 years</th>
<th>CS-IgE+</th>
<th>FHA+</th>
<th>CS-IgE+ and FHA+</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE</td>
<td>SP</td>
<td>PPV</td>
<td>LR+ SE</td>
</tr>
<tr>
<td>Allergic symptoms</td>
<td>26</td>
<td>84</td>
<td>64 1.6</td>
</tr>
<tr>
<td>SPT† positivity</td>
<td>24</td>
<td>82</td>
<td>61 1.3</td>
</tr>
<tr>
<td>Verified atopy‡</td>
<td>26</td>
<td>82</td>
<td>48 1.4</td>
</tr>
</tbody>
</table>

† Skin prick test
‡ Allergic symptoms and SPT positivity
Figure 8. Prevalence of atopic manifestations at age 20 years in subjects with CS-IgE $\geq 0.5$ kU/L and positive family history of allergy (FHA+) as compared to subjects with CS-IgE $< 0.5$ kU/L or negative family history of allergy (FHA-) (Study II, Pesonen 2008). AD, atopic dermatitis; ARC, allergic rhinoconjunctivitis; RW, recurrent wheezing; SPT, skin prick test positivity; VA, verified atopy (i.e. allergic symptoms and SPT positivity), totIgE+, an elevated total IgE ($\geq 130$ kU/L). Significant differences marked with asterisks (* $p < 0.03$, ** $p < 0.01$).
Inverse association between the retinol concentrations in infancy and subsequent atopic manifestations

The microbe-induced gut immune response in early infancy is assumed to be important in maturing the peripheral immune response (Noverr 2004). Retinoic acid is known to be essential in imprinting the gut tropism on T cells (Iwata 2004). Since an impaired gut immune response in infancy might contribute to the development of allergic sensitization, we decided to look for an association of plasma retinol concentrations in infancy and subsequent development of allergic symptoms.

The plasma retinol concentrations in cord blood (n = 97) and during the first year of life (n = 95) are presented in Figure 9 (III). The plasma retinol concentrations in infancy were lower in subjects who subsequently developed atopic manifestations in childhood and adolescence than in those who remained symptom-free. The difference in the retinol concentrations was most striking at 2 months of age.

Figure 9. Mean plasma retinol concentration during the first year of life. Shaded area, ± SD (Study III, Pesonen 2007).

At birth, the difference in plasma retinol concentrations between the subjects with and without subsequent atopic manifestations was not yet significant. However, the subjects with allergic symptoms, positive SPT or verified atopy (i.e. allergic symptoms and positive SPT) at age 5 years as well as those with an elevated total IgE
at age 20 years tended to have a lower cord blood retinol concentration than the nonatopic subjects.

The retinol concentration at age 2 months was inversely associated with the subsequent atopic manifestations in childhood and adolescence (Tables 5 and 6). The 14 children with positive SPT at age 5 years had a significantly lower retinol concentration at age 2 months than the SPT-negative children (p = 0.02). The retinol concentration at age 2 months tended to be lower in subjects with allergic symptoms and verified atopy at ages of 5 and 11 years. Symptoms of food hypersensitivity at ages of 5 (n = 10) and 11 years (n = 9) were associated with a comparatively low retinol concentration at age 2 months (p = 0.05 and 0.04, respectively). Serum total IgE elevated to 130 kU/L at age 11 years was associated with a low retinol concentration at age 2 months (p = 0.05), whereas there was no significant association of total IgE levels at ages of 5 and 20 and retinol concentrations in infancy.

**Table 5.** Retinol concentration (µg/L) at age 2 months (mean ± SD) and allergic symptoms, skin prick test (SPT) positivity and verified atopy at ages of 5 and 11 years (Study III, Pesonen 2007).

<table>
<thead>
<tr>
<th>Plasma retinol at age 2 months</th>
<th>Atopics</th>
<th>Controls**</th>
<th>P (t test)</th>
<th>P (adjusted)†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcomes at age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPT positivity</td>
<td>193 ± 41.6 (n=14)</td>
<td>223 ± 45.0 (n=71)</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Any allergic symptom</td>
<td>205 ± 47.1 (n=19)</td>
<td>222 ± 45.6 (n=64)</td>
<td>0.14</td>
<td>0.64</td>
</tr>
<tr>
<td>Verified atopy*</td>
<td>198 ± 47.8 (n=7)</td>
<td>220 ± 45.6 (n=77)</td>
<td>0.20</td>
<td>0.44</td>
</tr>
<tr>
<td><strong>Outcomes at age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPT positivity</td>
<td>212 ± 48.1 (n=32)</td>
<td>222 ± 43.0 (n=60)</td>
<td>0.27</td>
<td>0.21</td>
</tr>
<tr>
<td>Any allergic symptom</td>
<td>212 ± 41.5 (n=39)</td>
<td>222 ± 47.5 (n=51)</td>
<td>0.31</td>
<td>0.66</td>
</tr>
<tr>
<td>Verified atopy*</td>
<td>209 ± 39.7 (n=25)</td>
<td>221 ± 46.9 (n=65)</td>
<td>0.29</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* Verified atopy: subjects with an allergic symptom and positive SPT
** Controls: symptom-free/skin prick test negative subjects
† Adjusted for gender, duration of exclusive breast-feeding (< 2 or ≥ 2 months), smoking in the household during the first year, and family history of allergy.
Table 6. Retinol concentration (µg/L) at age 2 months (mean ± SD) and allergic symptoms, skin prick test (SPT) positivity and verified atopy at age 20 years (Study III, Pesonen 2007).

<table>
<thead>
<tr>
<th>Outcomes at age 20 years</th>
<th>Atopics</th>
<th>Controls**</th>
<th>P (t test)</th>
<th>P (adjusted)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any allergic symptom</td>
<td>209 ± 42.0 (n=46)</td>
<td>236 ± 43.9 (n=39)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>SPT positivity</td>
<td>208 ± 43.8 (n=46)</td>
<td>237 ± 40.9 (n=39)</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>Verified atopy*</td>
<td>203 ± 40.4 (n=35)</td>
<td>235 ± 43.1 (n=50)</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>All. rhinoconjunctivitis</td>
<td>205 ± 41.9 (n=37)</td>
<td>236 ± 43.9 (n=39)</td>
<td>0.002</td>
<td>0.01</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>207 ± 34.2 (n=19)</td>
<td>236 ± 43.9 (n=39)</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Recurrent wheezing</td>
<td>198 ± 39.8 (n=13)</td>
<td>236 ± 43.9 (n=39)</td>
<td>0.01</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Verified atopy: subjects with an allergic symptom and positive SPT
** Controls: symptom-free/skin prick test negative subjects
† Adjusted for gender, duration of exclusive breast-feeding (< 2 or ≥ 2 months), family history of allergy, smoking in the household during the first year, and smoking at age 20 years.

Atopic manifestations at age 20 years, i.e. allergic symptoms (n = 46), positive SPT (n = 46) and verified atopy (n = 35), were associated with a low retinol concentration at age 2 months (p = 0.01, 0.002 and 0.001, respectively; Table 6, Figures 10 and 11). The difference in the retinol concentrations was more pronounced in girls than in boys even though the boys had a higher frequency of allergic symptoms at age 20 years (56%) than the girls (50%). Subjects with allergic rhinoconjunctivitis (n = 37), recurrent wheezing (n = 13) and atopic dermatitis (n = 19) had a lower retinol concentration at age 2 months as compared with symptom-free subjects (p = 0.002, 0.01 and 0.01, respectively). All these associations remained significant after adjusting for the potential covariates, i.e. gender, family history of allergy, duration of exclusive breastfeeding, smoking in the household during the infant’s first year and smoking at age 20 years (Tables 5 and 6). Furthermore, the results remained unaltered after adjusting for maternal educational level, maternal age, sibship size and daycare attendance during the infant’s first year.

After the age of 2 months, the difference in retinol concentration in subjects with and without subsequent atopic manifestations became less significant. The retinol concentration at age 4 months was inversely associated with positive SPT and verified atopy at age 11 years (adjusted p = 0.03 and 0.02), and with atopic dermatitis at age 20 years (adjusted p = 0.04). Subjects with allergic symptoms, positive SPT or
verified atopy at age 5 years tended to have lower retinol concentrations at age 4 months as compared with symptom-free subjects. A similar trend was still observable at age 12 months, i.e. the retinol concentrations remained lower in subjects with subsequent atopic manifestations at ages of 5, 11 and 20 years than in those without atopy.

Figure 10. Plasma retinol in subjects with and without allergic symptoms at age 20 years (± SEM). * p = 0.01 (Study III, Pesonen 2007).
Figure 11. Plasma retinol in infancy in subjects with and without a positive skin prick test at age 20 years (± SEM). * p = 0.002 (Study III, Pesonen 2007).

Retinol concentrations in childhood and atopic manifestations

At age 5 years, the children with atopic dermatitis (n = 26) had a lower retinol concentration, 297 µg/L, than the symptom-free subjects, 322 µg/L, (p = 0.03). Furthermore, the children with allergic rhinoconjunctivitis, recurrent wheezing or food hypersensitivity and those with positive SPT at age 5 years had a lower retinol concentration than the children without atopic manifestations. At age 11 years, the plasma retinol concentrations were similar in children with and without atopic manifestations at that age. However, the subjects with allergic rhinoconjunctivitis (n = 59) or atopic dermatitis (n = 31) at age 20 years had a significantly lower retinol concentration at age 11 years as compared to those who remained symptom-free (p = 0.03 and 0.01, respectively).
Lower neonatal retinol concentrations in boys than in girls

We found that the plasma retinol concentrations were significantly lower in boys than in girls at birth and at age 2 months, whereas they were comparable from age 4 months onward (Table 7, III). Retinol concentrations in infancy and childhood tended to be lower in children with a family history of allergy than in those without, but the difference was not significant. Moreover, maternal atopy did not have an effect on the retinol concentrations in infancy. The infants with an elevated CS-IgE (II) had a lower mean plasma retinol in cord blood than the infants with CS-IgE below 0.5 kU/L, however, the difference was not statistically significant.

Table 7. Plasma retinol concentrations (µg/L, mean ± SEM) of the total population, and in girls and boys (Study III, Pesonen 2007).

<table>
<thead>
<tr>
<th>Age</th>
<th>Total P-retinol</th>
<th>Girls N</th>
<th>Girls P-retinol</th>
<th>Boys N</th>
<th>Boys P-retinol</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 months</td>
<td>222 (±5.7)</td>
<td>55</td>
<td>240 (±8.0)</td>
<td>42</td>
<td>197 (±6.3)</td>
<td>0.0001</td>
</tr>
<tr>
<td>2 months</td>
<td>218 (±4.6)</td>
<td>52</td>
<td>226 (±5.8)</td>
<td>43</td>
<td>208 (±7.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>4 months</td>
<td>254 (±4.6)</td>
<td>54</td>
<td>253 (±6.1)</td>
<td>41</td>
<td>256 (±7.1)</td>
<td>0.74</td>
</tr>
<tr>
<td>12 months</td>
<td>341 (±7.4)</td>
<td>54</td>
<td>347 (±9.3)</td>
<td>41</td>
<td>334 (±12.1)</td>
<td>0.32</td>
</tr>
<tr>
<td>5 years</td>
<td>320 (±4.6)</td>
<td>86</td>
<td>320 (±5.8)</td>
<td>69</td>
<td>319 (±7.4)</td>
<td>0.82</td>
</tr>
<tr>
<td>11 years</td>
<td>384 (±5.3)</td>
<td>94</td>
<td>385 (±6.8)</td>
<td>57</td>
<td>383 (±8.5)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

* P value calculated for the difference in retinol concentrations between girls and boys.
Inverse association between the cholesterol concentration and subsequent atopic manifestations

Cholesterol concentrations during the first year of life and subsequent allergic symptoms

Previous studies have indicated that a low level of serum cholesterol may be associated with allergic symptoms (Joki 2003, Shenoi 1992, Schäfer 2003). Therefore, we chose to examine whether the serum lipoprotein profile in infancy and childhood is associated with subsequent atopic manifestations.

We observed that children and adolescents with clinical and laboratory manifestations of atopy had lower cholesterol concentrations throughout infancy, childhood and adolescence as compared to nonallergic subjects (IV). Interestingly, this difference was not detectable in cord blood, but already became significant at age 2 months, when virtually all the infants were on a similar diet, i.e. on human milk feeding. The serum cholesterol and lipoprotein levels during the first year of life and the tracking of serum cholesterol and lipoproteins from birth to age 11 years in the present birth cohort have been previously published (Kallio 1992, Kallio 1998).

Cholesterol concentrations in cord blood did not differ significantly in subjects with and without subsequent allergic symptoms, skin prick test positivity or elevated total IgE at ages of 5, 11 and 20 years. The 14 (7%) children who had clinical signs suggestive of atopy during the first year of life (Savilahti 1987) had lower cholesterol concentrations throughout infancy as compared with the symptom-free infants. The mean cord blood cholesterol concentration was lower in infants with an elevated cord serum IgE (CS-IgE) as compared to those with CS-IgE below 0.5 kU/L, however, the difference was not significant.

Cholesterol concentrations in infancy and childhood are inversely associated with allergic symptoms at 5 years of age

The 40 children with allergic symptoms at 5 years of age had lower cholesterol concentrations in infancy and at age 5 years than the symptom-free children (Tables 8 and 9). The difference was significant at ages 2, 4 and 6 months, and at 5 years (p = 0.01, 0.04, 0.01 and 0.04, respectively). At age 2 months, nearly all the infants were on a similar diet, i.e. 94% of them were being breast-fed, so the finding was not dependent on differences in the diet. Most of the children with allergic symptoms at age 5 years had atopic dermatitis or symptoms of food hypersensitivity or a combination of these. The children with atopic dermatitis had lower cholesterol
concentrations at ages of 2 and 6 months \( (p = 0.02) \) and 5 years \( (p = 0.01, \) Tables 8 and 9) than the symptom-free children. Children with an elevated total IgE \( (\geq 48 \text{ kU/L}) \) had lower cholesterol concentrations in infancy than the children with total IgE below 48 kU/L, however, the differences were not statistically significant.

Table 8. Mean serum total cholesterol (mmol/L, ± SD) in infancy and the prevalence of allergic symptoms at age 5 years (Study IV, Pesonen 2007).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>9 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom-free ( (n=123) )</td>
<td>3.77 ±0.78</td>
<td>3.82 ±0.87</td>
<td>3.98 ±0.84</td>
<td>4.35 ±0.84</td>
<td>4.54 ±0.77</td>
</tr>
<tr>
<td>Atopic dermatitis ( (n=28) )</td>
<td>3.38 ±0.64</td>
<td>3.49 ±0.69</td>
<td>3.62 ±0.86</td>
<td>4.10 ±0.71</td>
<td>4.31 ±0.67</td>
</tr>
<tr>
<td>P*</td>
<td>0.02</td>
<td>0.08</td>
<td>0.02</td>
<td>0.09</td>
<td>0.40</td>
</tr>
<tr>
<td>Food hypersensitivity ( (n=19) )</td>
<td>3.27 ±0.88</td>
<td>3.61 ±0.70</td>
<td>3.57 ±0.84</td>
<td>4.21 ±0.75</td>
<td>4.28 ±0.63</td>
</tr>
<tr>
<td>P*</td>
<td>0.01</td>
<td>0.20</td>
<td>0.01</td>
<td>0.28</td>
<td>0.39</td>
</tr>
<tr>
<td>All. rhinoconjunctivitis ( (n=7) )</td>
<td>3.63 ±0.75</td>
<td>3.31 ±0.42</td>
<td>3.08 ±0.97</td>
<td>3.71 ±0.60</td>
<td>4.17 ±0.59</td>
</tr>
<tr>
<td>P*</td>
<td>0.63</td>
<td>0.15</td>
<td>0.01</td>
<td>0.07</td>
<td>0.39</td>
</tr>
<tr>
<td>Recurrent wheezing ( (n=5) )</td>
<td>3.00 ±1.13</td>
<td>3.14 ±0.31</td>
<td>3.24 ±1.07</td>
<td>4.32 ±1.10</td>
<td>4.26 ±0.90</td>
</tr>
<tr>
<td>P*</td>
<td>0.02</td>
<td>0.09</td>
<td>0.04</td>
<td>0.94</td>
<td>0.43</td>
</tr>
<tr>
<td>Any allergic symptom ( (n=40) )</td>
<td>3.40 ±0.78</td>
<td>3.52 ±0.43</td>
<td>3.60 ±0.77</td>
<td>4.24 ±0.81</td>
<td>4.36 ±0.68</td>
</tr>
<tr>
<td>P*</td>
<td>0.01</td>
<td>0.04</td>
<td>0.01</td>
<td>0.26</td>
<td>0.49</td>
</tr>
</tbody>
</table>

* P values calculated for the difference between the mean cholesterol levels of the symptomatic and the symptom-free children and adjusted for gender, exclusive breastfeeding for \( \geq 6 \) months, family history of allergy, and smoking in the household during the infant’s first year.
The LDL cholesterol concentration at age 5 years was significantly lower in the children with allergic symptoms than in the symptom-free children (Table 9). The HDL cholesterol concentration was similar in allergic and nonallergic children with the exception of the five children with recurrent wheezing, who had higher mean HDL cholesterol than the symptom-free children.

Table 9. Mean serum total, HDL and LDL cholesterol (mmol/L, ± SD) and allergic symptoms at age 5 years (Study IV, Pesonen 2007).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Total cholesterol</th>
<th>HDL</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom-free (n=123)</td>
<td>4.95 (±0.74)</td>
<td>1.49 (±0.27)</td>
<td>3.20 (±0.68)</td>
</tr>
<tr>
<td>Atopic dermatitis (n=28)</td>
<td>4.53 (±0.70)</td>
<td>1.52 (±0.40)</td>
<td>2.78 (±0.57)</td>
</tr>
<tr>
<td>P*</td>
<td>0.009</td>
<td>0.68</td>
<td>0.003</td>
</tr>
<tr>
<td>Food hypersensitivity (n=19)</td>
<td>4.61 (±0.69)</td>
<td>1.51 (±0.39)</td>
<td>2.86 (±0.55)</td>
</tr>
<tr>
<td>P*</td>
<td>0.06</td>
<td>0.79</td>
<td>0.03</td>
</tr>
<tr>
<td>Recurrent wheezing (n=5)</td>
<td>4.54 (±0.26)</td>
<td>1.79 (±0.29)</td>
<td>2.59 (±0.44)</td>
</tr>
<tr>
<td>P*</td>
<td>0.23</td>
<td>0.017</td>
<td>0.04</td>
</tr>
<tr>
<td>Any allergic symptom (n=40)</td>
<td>4.67 (±0.66)</td>
<td>1.57 (±0.38)</td>
<td>2.86 (±0.57)</td>
</tr>
<tr>
<td>P*</td>
<td>0.04</td>
<td>0.18</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* P values calculated for the difference between the mean cholesterol levels of the symptomatic and the symptom-free children.

Total IgE at age 11 years is inversely associated with cholesterol concentrations in infancy and childhood

Children with an elevated total IgE (≥ 130 kU/L, n = 31) had consistently lower total cholesterol concentrations throughout infancy and childhood than those with total IgE below 130 kU/L, and there was a significant difference in the cholesterol concentrations at ages 2, 4, 6, and 12 months (p = 0.04, 0.01, 0.05 and 0.01, respectively) and at 5 years (p = 0.05). These associations remained significant after adjusting for potential covariates. However, allergic symptoms at age 11 years were not associated with alterations in total, LDL or HDL cholesterol at that age.
Atopic manifestations at age 20 years are inversely associated with cholesterol concentrations in infancy, childhood and adolescence

The 85 subjects with allergic symptoms at 20 years of age had lower cholesterol concentrations throughout repeated measurements in infancy and childhood as compared to the symptom-free subjects (Figure 12). The differences in cholesterol concentrations at ages 9 and 12 months (p = 0.01 and 0.002, respectively) were significant after adjusting for potential covariates (Table 10). Furthermore, subjects who were skin-prick-test positive at age 20 years had lower cholesterol concentrations in infancy, childhood and adolescence as compared with the skin-prick-test negative subjects. The difference was significant at ages 4 and 12 months, and 5 years (Table 10).

Figure 12. Serum total cholesterol (± SEM) in subjects with and without allergic symptoms at age 20 years. M, months, y, years. Significant differences marked with an asterisk (Study IV, Pesonen 2007).
Table 10. The association of serum cholesterol concentrations and subsequent atopic manifestations at age 20 years as adjusted for potential covariates* (Study IV, Pesonen 2007).

<table>
<thead>
<tr>
<th></th>
<th>OR (95%CI)†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol at age 4 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin prick test positivity at age 20 years</td>
<td>0.62 (0.39-0.96)</td>
<td>0.04</td>
</tr>
<tr>
<td>Cholesterol at age 6 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgE ≥ 130kU/L at age 20 years</td>
<td>0.49 (0.28-0.84)</td>
<td>0.01</td>
</tr>
<tr>
<td>Cholesterol at age 9 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any allergic symptom at age 20 years</td>
<td>0.57 (0.36-0.91)</td>
<td>0.02</td>
</tr>
<tr>
<td>Atopic dermatitis at age 20 years</td>
<td>0.36 (0.18-0.73)</td>
<td>0.01</td>
</tr>
<tr>
<td>IgE ≥ 130kU/L at age 20 years</td>
<td>0.55 (0.31-0.95)</td>
<td>0.03</td>
</tr>
<tr>
<td>Cholesterol at age 12 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any allergic symptom at age 20 years</td>
<td>0.46 (0.29-0.76)</td>
<td>0.002</td>
</tr>
<tr>
<td>Allergic rhinoconjunctivitis at age 20 years</td>
<td>0.41 (0.23-0.73)</td>
<td>0.002</td>
</tr>
<tr>
<td>Skin prick test positivity at age 20 years</td>
<td>0.54 (0.33-0.90)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cholesterol at age 5 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin prick test positivity at age 20 years</td>
<td>0.59 (0.34-1.01)</td>
<td>0.05</td>
</tr>
<tr>
<td>IgE ≥ 130kU/L at age 20 years</td>
<td>0.41 (0.21-0.77)</td>
<td>0.01</td>
</tr>
<tr>
<td>Cholesterol at age 11 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any allergic symptom at age 20 years</td>
<td>0.47 (0.25-0.88)</td>
<td>0.02</td>
</tr>
<tr>
<td>Allergic rhinoconjunctivitis at age 20 years</td>
<td>0.43 (0.21-0.89)</td>
<td>0.02</td>
</tr>
<tr>
<td>IgE ≥ 130kU/L at age 20 years</td>
<td>0.40 (0.18-0.90)</td>
<td>0.03</td>
</tr>
<tr>
<td>Cholesterol at age 20 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgE ≥ 130kU/L at age 20 years</td>
<td>0.56 (0.33-0.96)</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL cholesterol at age 5 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic rhinoconjunctivitis at age 20 years</td>
<td>0.50 (0.25-0.97)</td>
<td>0.04</td>
</tr>
<tr>
<td>IgE ≥ 130kU/L at age 20 years</td>
<td>0.32 (0.15-0.68)</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL cholesterol at age 11 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent wheezing at age 20 years</td>
<td>0.25 (0.07-0.87)</td>
<td>0.03</td>
</tr>
<tr>
<td>Allergic rhinoconjunctivitis at age 20 years</td>
<td>0.40 (0.18-0.86)</td>
<td>0.02</td>
</tr>
<tr>
<td>IgE ≥ 130kU/L at age 20 years</td>
<td>0.37 (0.16-0.84)</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL cholesterol at age 20 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgE ≥ 130kU/L at age 20 years</td>
<td>0.48 (0.26-0.89)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Multivariate logistic regression. Independent variables used in the model: Gender, exclusive breastfeeding for ≥ 6 months, family history of allergy, smoking in the household during the infant’s first year and smoking status of the subject at age 20 years.
† Odds ratio and 95% confidence interval
Total IgE $\geq 130$ kU/L at age 20 years (n = 42) was associated with decreased cholesterol concentrations throughout infancy, childhood and adolescence (Figure 13). The associations between IgE $\geq 130$ kU/L and cholesterol at ages 6 and 9 months, and 5, 11 and 20 years were significant after adjusting for potential covariates (Table 10).

Subjects with IgE $\geq 130$ kU/L had a lower LDL cholesterol concentration at 5, 11 and 20 years of age ($p = 0.001$, 0.02 and 0.02, respectively) as compared to subjects with IgE < 130 kU/L. Furthermore, low LDL cholesterol at age 11 years was associated with subsequent allergic rhinoconjunctivitis ($p = 0.02$) and recurrent wheezing ($p = 0.03$) at age 20 years (Table 10).

Figure 13. Serum total cholesterol ($\pm$ SEM) in subjects with an elevated and a normal total IgE at age 20 years. M, months, y, years. Significant differences marked with an asterisk (Study IV, Pesonen 2007).

We found that the retinol and also the cholesterol concentrations at age 2 months were inversely associated with subsequent atopic manifestations (III, IV). The possible interaction of the retinol and cholesterol concentrations was examined by logistic regression analyses (Table 11). All the associations of plasma retinol at age 2 months and atopic outcomes, i.e. allergic symptoms, SPT positivity and verified atopy at age
20 years, and SPT positivity at age 5 years (Tables 5 and 6), remained significant after adjusting for the serum cholesterol concentration. Furthermore, there was no statistically significant correlation between the serum cholesterol and the plasma retinol concentrations at age 2 months (Spearman’s rank test, \( p = 0.17, \rho = 0.1 \)). A similar analysis for the outcomes with a significant inverse association with the cholesterol concentration was not feasible due to the limited number of subjects in whose both the retinol and the cholesterol concentrations in infancy had been measured. Therefore, although we did not find evidence of an interaction between the retinol and the cholesterol concentrations, this possibility cannot be excluded.

**Table 11.** The inverse association of plasma retinol at age 2 months and subsequent atopic manifestations at ages 5 and 20 years* as adjusted for serum cholesterol.

<table>
<thead>
<tr>
<th>Outcome at age 5 years</th>
<th>OR (95% CI)†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPT positivity</td>
<td>0.98 (0.96-0.99)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcomes at age 20 years</th>
<th>OR (95% CI)†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic symptoms</td>
<td>0.98 (0.97-0.99)</td>
<td>0.01</td>
</tr>
<tr>
<td>SPT positivity</td>
<td>0.98 (0.97-0.99)</td>
<td>0.01</td>
</tr>
<tr>
<td>Verified atopy</td>
<td>0.98 (0.97-0.99)</td>
<td>0.004</td>
</tr>
<tr>
<td>Allergic rhinoconjunctivitis</td>
<td>0.98 (0.97-0.99)</td>
<td>0.01</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>0.98 (0.97-0.99)</td>
<td>0.04</td>
</tr>
<tr>
<td>Recurrent wheezing</td>
<td>0.98 (0.96-0.99)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Multivariate logistic regression. Serum cholesterol at age 2 months and family history of allergy were included as independent variables.
† Odds ratio and 95% confidence interval.
DISCUSSION

Setting of the study: Follow-up from birth to 20 years of age

This prospective 20-year follow-up study involves an unselected cohort of healthy Finnish newborns and mothers. Since the study was originally set up to examine the nutritional value of strictly exclusive breastfeeding, all the participants irrespective of atopic heredity were encouraged to maintain exclusive breastfeeding as long as it was nutritionally sufficient for the infant, and inadvertent early exposure to cow's milk was carefully avoided. Therefore, the potential bias of infants of allergic families being self-selected to receive a prolonged exclusive breastfeeding was avoided, and the infants with a family history of allergy and those without a family history of allergy were equally likely to receive a prolonged exclusive breastfeeding. The frequent follow-up during the infants' first year of life, skillfully and devotedly performed by the late pediatrician Leena Lope (former Salmenperä), enabled a reliable recording of the duration of exclusive breastfeeding as well as the repeated measurements of serum parameters. To the author's knowledge, the present study with its 20-year follow-up period represents the longest prospective follow-up of newborns on prolonged exclusive breastfeeding published to date.

The strengths of the present study also include the high participation rates during the first year and at the follow-up examinations at ages of 5, 11 and 20 years (82%, 76%, and 83%, respectively). As many as 90% of the eligible families agreed to participate, and only two families dropped out during the first year of follow-up. Since the cohort consists of randomly selected healthy newborns, the cohort may be considered representative of an urban Finnish population. However, the high frequency and duration of prolonged exclusive breastfeeding in the study cohort as compared to the general population (Hasunen 2005) must be taken into account when interpreting the results of the present study. The uniform mode of infant feeding may have accentuated or enabled the detection of differences in e.g. cholesterol and retinol levels between subjects with and without subsequent atopic manifestations, which might be less clear in infant population with more variable dietary choices.

At the follow-up examinations, the information on the occurrence of allergic symptoms were collected by personal interviews and physical examinations instead of postal questionnaires, and the interviewing physician was blinded for the early feeding regimen and the laboratory test results. In addition to information obtained by means of the interviews and clinical examination, also objective markers of atopy, i.e. SPT and serum total IgE, were used as outcomes. Instead of concentrating on a single aspect of atopy such as asthma or atopic dermatitis, an attempt was made to cover most of the symptoms considered as atopy-related, i.e. allergic rhinoconjunctivitis, recurrent wheezing, atopic dermatitis and symptoms of food hypersensitivity. This
approach offers the advantage that the subjects classified as nonatopic were symptom-free with regard to all and not just one of the allergic symptoms.

As a limitation of the study, the reports of recurrent wheezing were not confirmed with objective demonstration of bronchial hyperresponsiveness. Furthermore, although double-blind placebo-controlled food challenge is considered the golden standard of diagnosis of food allergy (Young 1994), performing these was beyond the scope of our resources. Instead, we chose to rely on the history of food hypersensitivity based on reports of typical symptoms appearing after ingestion of a specific food. At the age of 20 years, reports of food hypersensitivity symptoms in subjects without any other allergic symptom were considered as possibly non-atopic; therefore, these were not taken into account in the analyses.

Controversial effects of breastfeeding in allergy prevention

Breastfeeding is the preferred method of infant nutrition because of its nutritional, immunological and psychological benefits (Kramer 2001, American Academy of Pediatrics 1997). However, the role of breastfeeding in allergy prevention remains indefinite despite the large number of studies and publications aiming at clarifying the issue (Friedman 2005). One possible explanation for the controversy is that the methodological differences and weaknesses in study design have led to conflicting results. In retrospective studies, the maternal recall on the actual duration of exclusive breastfeeding may be inaccurate (Ruowei 2005). If the information on the mode of infant feeding and the duration and exclusivity of breastfeeding has been collected retrospectively, i.e. after the breastfeeding period and at a time when the child may already have developed symptoms of atopy, it is difficult to exclude the possibility of reverse causation (Bergmann 2002). The inconsistent results of studies on the allergy-preventive effect of breastfeeding may also be due to the properties of breast milk such as the immunological complexity and the allergens present in breast milk, which may in some cases be sensitizing and in other cases protective. Furthermore, the genetic differences among individuals may have an effect on whether breastfeeding would be sensitizing or protective. According to critical reviews and meta-analyses of the published data from 1966 to 2000-2001, exclusive breastfeeding for the first 3-4 months may be considered protective against childhood asthma and atopic dermatitis in children with a hereditary risk for atopy (van Odijk 2003, Gdalevich 2001 a, Gdalevich 2001 b). However, several studies have not been able to show a protective effect, and some studies suggest that breast-fed infants may have an increased risk of developing allergic symptoms as compared to formula-fed infants (Friedman 2005).

In our study cohort, all the infants received at least some duration of exclusive breastfeeding in early infancy, and 94% of the infants were on breastfeeding at age 2 months. Therefore, the present study cannot address the question of whether breastfeeding of any duration confers an allergy-preventive effect as compared to never having been breast-fed, i.e. exclusive milk formula feeding. Instead, we were
able to examine the effects of a prolonged exclusive breastfeeding as compared to a shorter duration of exclusive breastfeeding, which is not a widely covered topic in previous literature.

The results of the present study (I) indicate that prolonging exclusive breastfeeding above the age of 6 months does not confer any additional benefit in allergy prevention. Instead, exclusive breastfeeding for 9 months or more was associated with increased atopic dermatitis and symptoms of food allergy in childhood in subjects with a family history of allergy. This somewhat unexpected finding lends support from several previous studies reporting increased frequency of atopic dermatitis in breast-fed subjects (Bergmann 2002, Purvis 2002, Miyake 2003). The reasons of the observed increase in the frequency of atopic dermatitis and food hypersensitivity symptoms in subjects having received exclusive breastfeeding for the first 9-12 months remain to be clarified. However, our results indicate that prolonging exclusive breastfeeding above the generally recommended age of 6 months does not confer any additional allergy-preventive effect, and exclusive breastfeeding prolonged to 9-12 months of age may increase the risk of subsequent allergic symptoms.

**CS-IgE as a possible marker of subsequent atopic manifestations up to age 20 years**

Early prevention is regarded as an important corner stone in the management of atopic diseases, and some new primary and secondary allergy preventive strategies involving the administration of probiotics, oral antihistamines and specific immunotherapy have recently been suggested (Kalliomäki 2003, Möller 2002, Warner 2001). Therefore, there is a need to find reliable, noninvasive and practicable predictors of atopy, which might identify infants at risk and allow the initiation of preventive strategies early in life (Allam 2005).

Most of the previous studies on the allergy-predictive capacity of CS-IgE have focused on the atopic manifestations in infancy or early childhood with the exception of two studies with a follow-up extended to 10-11 years of age (Croner 1990, Sadeghnejad 2004). Comparisons of the results of the previous studies are complicated by the variable cut-offs for an elevated CS-IgE and the different definitions of allergic outcomes. However, due to the low sensitivities reported, CS-IgE is considered ineffective as an exclusive predictive marker for subsequent allergy (Allam 2005).

We observed that an elevated CS-IgE was associated with atopic dermatitis, food hypersensitivity symptoms and SPT reactivity in children at age 5 years (II). Furthermore, an elevated CS-IgE was associated with allergic rhinoconjunctivitis at age 20 years, and with a high serum total IgE at ages of 11 and 20 years. Using a combination of a family history of allergy and an elevated CS-IgE in predicting subsequent atopic manifestations resulted in improved specificity and positive likelihood ratio, and a reduced sensitivity as compared to an elevated CS-IgE or a family history of allergy alone.
Because an exceptionally high CS-IgE value might be due to contamination with maternal blood, we excluded from further analyses one newborn with CS-IgE value above 10 kU/L. Alternatively, given that the maternal IgA does not cross the placenta, and IgA concentration in cord blood is known to be low (Haworth 1966, Weemaes 2003, Monteiro 2003), the maternal contamination of cord blood might be excluded by measuring the cord blood IgA and excluding subjects with a high IgA in cord blood (Ownby 1996).

The cut-off level we used, 0.5 kU/L, has been found optimal in predicting atopic outcomes in two previous studies (Sadeghnejad 2004, Tariq 1999). In another study using the same cut-off level and a similar CS-IgE determination method (PRIST®), the specificities of CS-IgE and the combination of CS-IgE and family history of allergy were very close to those we found, whereas the sensitivities were higher in our study (Hansen 1993). These differences may in part be due to the fact that in the previous study, 'allergy at age 5 years' was defined as the presence of atopic symptoms and a high serum IgE, whereas we defined 'verified atopy' as the presence of atopic symptoms and a positive SPT.

Based on our results and those of the previous studies, the measurement of CS-IgE as a screening test for atopic predisposition cannot be recommended for the general population due to the low sensitivity of CS-IgE. However, combined with the family history of allergy it may be used in identifying infants with a high risk of subsequent atopic manifestations (Zeiger 1995). This may be useful in future, with the emergence of new allergy-preventive strategies, which are to be directed to subjects at a high risk of developing allergic diseases (Kalliomäki 2003, Möller 2002, Warner 2001).

Association of vitamin A concentration and atopic manifestations

The gut immune response, induced by the microbial antigens (Hooper 2004, Rhee 2004), is assumed to play an important role in maturing the infant's peripheral immune response (Macpherson 2004, Noverr 2004). Retinoic acid is essential to the imprinting of the gut-homing specificity on T cells (Iwata 2004), and enhances the induction of regulatory T cells by the dendritic cells of the gut-associated lymphoid tissue (von Boehmer 2007). Previously, children with asthma have been reported to have lower concentrations of serum vitamin A and β-carotene than healthy controls (Arora 2002, Rubin 2004). Furthermore, a low intake of retinol in infancy has been associated with childhood atopic eczema in a follow-up study on children with a family history of allergy (Laitinen 2005).

We had the opportunity to explore the eventual association of the plasma retinol levels in infancy and the subsequent development of atopic manifestations in childhood and adolescence. In our study (III), the plasma retinol concentration at age 2 months was inversely associated with the subsequent development of allergic symptoms and positive skin prick test in childhood and at age 20 years. Furthermore, the retinol concentration at age 5 years was inversely associated with the prevalence
of atopic dermatitis at that age. Dietary vitamin A deficiency was unlikely, since all the infants of our study cohort received vitamin A supplementation. The maternal blood vitamin A concentrations during pregnancy were not monitored in our study. However, the mean retinol concentration of cord blood was similar than the values previously reported from European populations (Sapin 2000, Schulpis 2004).

In our study, the differences in the retinol concentrations were observable at age 2 months when nearly all the infants were on a similar diet, i.e. on breastfeeding. Thus, we suggest that the differences in the retinol concentrations between the atopic and the nonatopic subjects are not dependent of the diet; instead, the retinol concentration is subject to genetic control, in which there may be atopy-related differences. We hypothesize that a low concentration of retinol in an infant with atopic predisposition may interfere with the T-cell homing to the gut and thereby disturb the development of normal T helper type 1 responses elicited by the gut microflora. In future studies, it might be interesting to examine the possible differences in the expression of the gut-homing mediators on the intestinal T cells in young infants with and without subsequent allergic diseases, and whether the expression is related to serum retinol concentrations.

Inverse association of serum cholesterol levels in infancy and subsequent atopic manifestations in childhood and adolescence

The increase in the prevalence of allergic diseases in industrialized countries has been suggested to be linked to an altered dietary consumption of polyunsaturated fatty acids (Kankaanpää 1999). Based on several studies which demonstrate an altered dietary and serum polyunsaturated fatty acid composition with higher levels of n-6 fatty acids in atopic subjects (Dunder 2001, Leichsenring 1995, Pöysä 1991), an increased intake of n-6 polyunsaturated fatty acids and a decreased intake of n-3 fatty acids have been suggested to have contributed to the increase of allergic diseases in the industrialized countries (Black 1997). Serum cholesterol level is increased by a high intake of saturated fatty acids (Grundy 1988), whereas linoleic acid, the major dietary n-6 polyunsaturated fatty acid, has an opposite effect (Mensink 1987). Therefore, if the increased intake of n-6 fatty acids is causative for allergic diseases, total cholesterol may be expected to be inversely associated with atopic manifestations (Schäfer 2003). This led us to explore the possible association of cholesterol levels and atopic manifestations in the setting of the present follow-up cohort study.

We found that the cholesterol levels of the subjects with subsequent atopic manifestations were significantly lower already in early infancy, at a time when most of the infants of our study cohort were still on exclusive breastfeeding (IV). Therefore, our results suggest that the differences in cholesterol levels between atopic and nonatopic subjects are not explained by differences in diet. In support of this view is the recent finding that the modification of dietary polyunsaturated fatty acids in early childhood was not effective in preventing atopy and asthma, even though the
dietary intervention had a clear effect on the plasma $n$-3 and $n$-6 fatty acid levels (Almqvist 2007).

Our finding that the inverse association between the cholesterol concentration and subsequent atopic manifestations was present as early as at 2 months of age suggests that a decreased cholesterol level may contribute to the early steps in the pathogenesis of allergic sensitization. Once the atopic constitution is established, the association of atopic manifestations and serum lipid levels may be less significant. This hypothesis is supported by the observations that a low serum cholesterol level is associated with food allergy and respiratory allergy in children, but the association is not significant in adults (Joki 2003, Shenoi 1992, Schäfer 2003).

Since both the cholesterol and the retinol concentrations in infancy were found to be inversely associated with subsequent atopic manifestations, there might be an interaction of the cholesterol and the retinol concentrations in infancy. We failed to find evidence of such an interaction; however, this may be due to the limited number of subjects available for analysis, given that the plasma retinol concentrations in infancy were measured only in approximately half of the study population.

It remains to be clarified in future studies, whether our finding of lower cholesterol levels in infancy in subjects with subsequent atopy can be reproduced in populations without an intervention promoting prolonged exclusive breastfeeding. Another interesting aim of the future studies is to find out whether the difference in cholesterol levels between atopic and nonatopic subjects persists later in life, i.e. beyond the age of 20 years. If this is the case, the somewhat lower serum cholesterol concentration of the atopic subjects might possibly provide an advantage with respect to the prevention of atherosclerosis and coronary heart disease. However, the previous literature is not in support of this hypothesis, since in the Helsinki Heart Study, a high serum IgE level in middle-aged dyslipidemic men was not protective against atherosclerosis but instead, was suggested to be a risk factor for myocardial infarction (Kovanen 1998).
CONCLUSIONS

The conclusions based on the results of this thesis are as follows:

Prolonging strictly exclusive breastfeeding above the generally recommended 6 months of age does not confer additional benefit in allergy prevention. On the contrary, in infants with a family history of allergy, exclusive breastfeeding prolonged for 9 months or above is associated with increased subsequent atopic dermatitis and food hypersensitivity symptoms in childhood.

Elevated CS-IgE in newborns is associated with subsequent atopic manifestations in children and up to age 20 years. A combination of an elevated CS-IgE and a positive family history of allergy predicted atopic manifestations with a higher positive likelihood ratio and specificity than the CS-IgE level or the family history of allergy alone. By using the combination of family history of allergy and CS-IgE as a criterion, it is possible to identify a group of infants who are very likely to develop allergic symptoms later in life. This may be useful in targeting allergy-preventive measures to infants at a high risk of developing allergic symptoms.

Retinol and cholesterol concentrations in infancy are identified as early determinants of subsequent atopic manifestations. The plasma retinol concentration at age 2 months is inversely associated with the subsequent development of allergic symptoms and atopic sensitization in childhood and adolescence in an affluent urban population, in which dietary vitamin A deficiency is unlikely. Children and adolescents with allergic symptoms, skin prick test positivity and an elevated IgE have lower total cholesterol levels in infancy and childhood than the nonatopic subjects. The decrease in total cholesterol levels appears to be due to a decreased low-density lipoprotein cholesterol (LDL) concentration in the atopic subjects. Since the difference in cholesterol concentrations is detectable as early as from age 2 months onward, it is unlikely to be due to atopy-related dietary alterations.

These findings suggest that there may be atopy-related inborn features of retinol and lipoprotein metabolism, which result in decreased retinol and LDL cholesterol levels in infants and children with atopic predisposition.
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Maria Pesonen
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