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# **LIFESTYLE, SALIVARY CORTISOL AND ALLERGY IN CHILDREN**

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## ABSTRACT

**Background:** IgE-mediated allergic diseases (eczema, food allergy, asthma and rhinoconjunctivitis) are common chronic diseases in childhood. In Sweden 30-40% of the children are affected by allergic manifestations during childhood. The understanding and prevention of allergic diseases require further studies of the determinants of these diseases. Exposure to environmental factors before birth or early in life is most likely responsible for the comparatively rapid changes in prevalence. The anthroposophic lifestyle has previously been associated with a low prevalence of allergic diseases in children. Psychosocial factors and the stress-related hormone cortisol have been associated to allergic diseases.

**Aim:** The overall aim of this thesis was to investigate patterns of lifestyle early in life in relation to salivary cortisol levels in infancy and allergic sensitization and disease during childhood.

**Methods:** The material in this thesis is based on the ALADDIN (Assessment of Lifestyle and Allergic Disease During Infancy)-study where 330 families with different lifestyles were recruited during pregnancy. Demographic and exposure data were obtained by questionnaires and interviews. Information on allergy related symptoms was obtained by repeated examinations of the children. Salivary samples for analysis of cortisol were collected at six months of age on three occasions during one day from the children and their parents. Blood samples were collected from the parents before birth and from the children at birth and at six, twelve and 24 months of age for analyses of IgE.

**Results:** Many exposure characteristics before birth and early in life differed between children from families with an anthroposophic lifestyle in comparison to other children. The prevalence of IgE-sensitization to common food and inhalant allergens in children was lower among children from families with an anthroposophic lifestyle compared to other children during the first two years of life. Correlations were found between salivary cortisol levels in six-months-olds and their parents, for the mother on all three sampling occasions and for the father in the afternoon and evening. Children from families with an anthroposophic lifestyle had lower levels of salivary cortisol at six months of age compared to other children. Increasing levels of salivary cortisol at six months of age was associated to an increased prevalence of sensitization and eczema during the first two years of life.

**Conclusion:** Different lifestyle aspects had an influence on levels of salivary cortisol early in life. These levels were associated to allergic sensitization and symptoms in children. The results may increase knowledge about mechanisms behind undesired immunologic reactions in children. This is needed to enable prevention or early treatment of allergy, and thus improve living conditions for those affected today and tomorrow.

## **LIST OF ABBREVIATIONS**

ALADDIN	Assessment of Lifestyle and Allergic Disease During Infancy
CI	Confidence Interval
GEE	Generalized Estimating Equations
HPA-axis	Hypothalamus-Pituitary-Adrenal-axis
IgE	Immunoglobulin E
ISAAC	International Study of Asthma and Allergies in Childhood
kUa/L	Kilo units of allergens per liter
M-CHC	Maternal-Child Health Center
OR	Odds Ratio

## LIST OF PUBLICATIONS

- I. **Stenius F**, Swartz J, Lilja G, Borres M, Bottai M, Pershagen G, Scheynius A, Alm J. Lifestyle factors and sensitization in children – the ALADDIN birth cohort. Manuscript submitted.
- II. **Stenius F**, Theorell T, Lilja G, Scheynius A, Alm J, Lindblad F. Comparisons between salivary cortisol levels in six-months-olds and their parents. *Psychoneuroendocrinology* 2008; Apr:33(3): 352-359.
- III. **Stenius F**, Swartz J, Lindblad F, Pershagen G, Scheynius A, Alm J, Theorell T. Low salivary cortisol levels in infants of families with an anthroposophic lifestyle. *Psychoneuroendocrinology* 2010; Nov:35(10): 1431-7.
- IV. **Stenius F**, Borres M, Bottai M, Lilja G, Lindblad F, Pershagen G, Scheynius A, Swartz J, Theorell T, Alm J. Salivary Cortisol levels and allergy in children – the ALADDIN birth cohort. Manuscript submitted.

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# 1 INTRODUCTION

IgE-mediated allergic diseases (eczema, food allergy, asthma and rhinoconjunctivitis) are common chronic diseases in childhood (1). The prevalence is high and has increased in many parts of the world during the past decades (2-4). In Sweden 30-40 % of the children are affected by allergic manifestations during childhood (4, 5). Future prevention of chronic diseases in childhood is a possibility to decrease the effects on individuals and society (6). Preventive actions require further studies of the determinants of allergic disease (7, 8). Exposures to environmental factors before birth or early in life are most likely responsible for the comparatively rapid increase in prevalence of allergic diseases (9, 10). The anthroposophic lifestyle has previously been associated to a low prevalence of allergic diseases (11-13). Psychosocial factors and the stress-related hormone cortisol have been associated to allergic diseases (14-17).

The ALADDIN study is a prospective birth cohort study, which was initiated in 2004 to study the influence of specific lifestyle factors on the development of allergic diseases in childhood. This thesis is based on material from the ALADDIN study and focuses on lifestyle early in life in relation to salivary cortisol and the development of allergic sensitization and symptoms. The first paper describes several exposure factors early in life for children from families with an anthroposophic or conventional lifestyle and also assesses the levels of allergic sensitization in these children. The second paper includes analyses of salivary cortisol levels in infants at six months of age in relation to levels of salivary cortisol of their parents. In the third paper salivary cortisol levels early in life are studied in children from families with an anthroposophic lifestyle compared to other children. In the fourth paper salivary cortisol levels early in life are studied in relation to allergic sensitization and symptoms during the first two years of life.

## 2 BACKGROUND

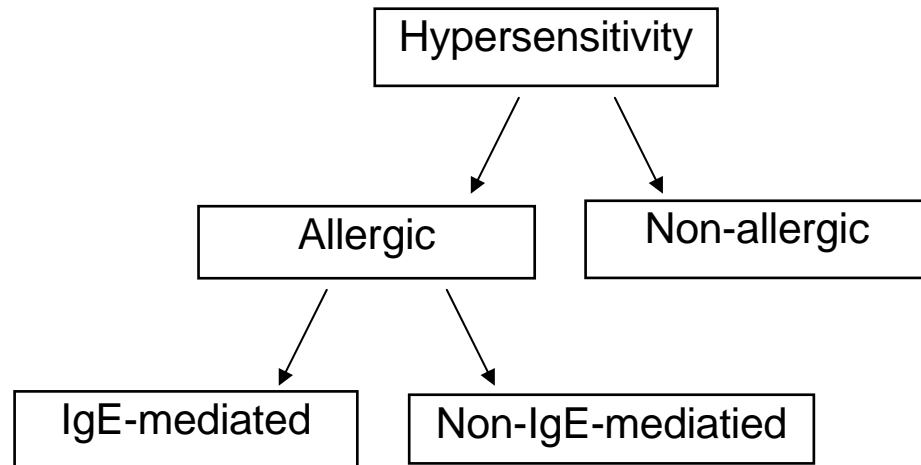
### 2.1 Historical perspective of allergy

Descriptions of symptoms like allergic reactions date back to ancient times and the ideas concerning the etiology to such diseases have changed over time (18). In 1819 the English physician John Bostock described perennial symptoms which seemed to be hay fever (19). This is assumed to be the first description in modern times of allergic symptoms, but the symptoms were not considered to be related to pollen, but more to physical factors, possible temperature (20). In the beginning of the 20<sup>th</sup> century it was finally proposed that allergic diseases rise from reactions to harmless environmental factors. The term allergy (from Greece *allos* “else, other” and *ergon* “effect, project”) meaning changed reactivity (21) was introduced in 1906 by Clarens Von Pirquet who described skin reactions to vaccines and denoted allergy to be a deviation from the original state of an organism due to its contact with an organic agent (22). The term atopy was introduced in the 1920’s by Coka and Cooke to describe the heredity syndrome of asthma and hay fever (23). The knowledge of the molecular basis of allergies developed during the 20<sup>th</sup> century, including the discovery of the IgE antibody in the 1960’s (24, 25). Even though now accepted that symptoms occur when the individual is exposed to different environmental agents, the epidemiology and etiology of allergic diseases is an important research topic and much remains to be disentangled.

### 2.2 Nomenclature

Symptoms and signs related to exposure to different harmless environmental stimuli are common in children (1). Many reactions are unspecific and should be differed from those mediated by an immune-response, usually by IgE or by T-cells (26, 27). To study the etiology of allergic diseases has been problematic due to inconsistent definitions in different studies and countries, and the need for a revised nomenclature has been obvious (28). Therefore an international collaboration of researchers has proposed a uniform nomenclature (28, 29) (fig1). The term for abnormal objectively and reproducible symptoms and signs initiated by exposure to a defined environmental stimulus is hypersensitivity (29). The defined stimulus should also be tolerated by the general population (29). If this reaction is mediated by specific immunologic mechanisms it is defined as an allergic hypersensitivity, more often known as allergy, and otherwise a non-allergic hypersensitivity (29). Allergic hypersensitivity (allergy) is classified as IgE-mediated or non-IgE mediated, the

later meaning mediated by other antibodies than IgE or cell mediated (figure 1). The term atopy is used for the tendency of a person to produce IgE antibodies in a high rate in response to ordinary exposures to allergens (29). A consequence can be symptoms like asthma, eczema or rhinoconjunctivitis, but the term atopy in it self does in this revised nomenclature not include clinical symptoms (29).



*Figure 1. Classification of hypersensitivity, modified from reference (28, 29).*

### **2.3 Sensitization and clinical symptoms**

The term sensitization is used to describe an elevated IgE level, commonly over 0,35 kU/L (30). Regarding clinical symptoms the term eczema is proposed to replace the previously used term atopic eczema/dermatitis (29). The clinical symptoms of allergy do not always follow the presence and levels of IgE (sensitization) (27). There is variation in the strength of the association between IgE sensitization and allergic symptoms but detection of allergen specific IgE seems to be associated with an increased risk of developing allergic respiratory disease later in life and with a more persistent and severe disease (31, 32).

The allergic symptoms differ at different ages during childhood and this progression of allergic manifestations is usually known as the atopic march (26, 32-34). The first manifestation is often eczema, a local inflammation of the skin with itching and redness that often affects the children during the first few months of life (7, 35). Also food allergies, especially to cow's milk or egg are common early in life. Later, in school-aged children, allergic rhinitis and allergic asthma are more common (7). However, this process is not true for all patients. A systematic review showed that only one of three children with eczema develops asthma later

during childhood (36). Still it seems to hold that different symptoms are more common in different ages during childhood, even though not always following the same sequence in every individual patient.

## **2.4 Prevalence**

The IgE-mediated allergic diseases are one of the most common chronic diseases in childhood (1, 4). The prevalence is high and has increased in many parts of the world during the past decades, especially in the Western world (2, 3). However, in areas where the prevalence is high there now appears to be stabilization (3, 37, 38). The prevalence differs in literature and is hard to determine due to geographic variations, diverging definitions and study design. In one study cow's milk protein allergy affected 2–7.5 % of children (39). Persistence in adulthood is uncommon since around 80% develop tolerance within 3–4 years (40). Eczema is the most common chronic skin disease during childhood and has in children without heredity for eczema been shown to have a prevalence of 27 % in children up to four years of age (41). In Sweden around 30-40 % of all children are affected by allergic manifestations during childhood (4, 5).

## **2.5 Prevention of allergic diseases**

Prevention is central for health care in children and has substantial impact for future health for the individual (6). The study of the determinants of allergic diseases in children is important for the possibilities for future prevention (8). These diseases have significant emotional and social effects for the children and their families and also effects and costs for the society (42). The immunological mechanisms behind the development of IgE-mediated allergies are complex and prevention strategies are therefore difficult to establish. Thus, allergies have been managed mainly by pharmacologic therapy, immunotherapy and for eczema skin care (6, 43). The treatment for cow's milk allergy has so far been strict avoidance of allergens, although other options may be possible in the future (40).

IgE-sensitization to an allergen is not always equivalent to having symptoms (31, 32). The term primary prevention of allergic diseases usually involves prevention of sensitization, and secondary prevention to avoid symptoms in a person who is sensitized (7). These definitions are although not clearly separated since sensitization and symptoms are not always clearly related (31, 32). To study the determinants of allergic diseases could give insight into the possible and important prevention of these diseases, which

could hypothetically decrease the prevalence, costs and burden of allergic diseases (44-46).

## **2.6 Environmental factors**

The determinants of IgE-mediated allergic diseases include interplay between genetic and environmental factors and the timing of these exposures (1). The influence of genetics on allergic diseases is strong (47), but genetic factors can not explain the rapid increases in prevalence or the regional differences in prevalence seen in regions with similar ethnic background (10). The expression of some of the genes may principally be influenced by environmental exposures (epigenetics) (48-50). Factors in the environment are not only triggers of allergic symptoms but also involved in the etiology of allergic diseases (10, 51, 52).

In 1989 the English epidemiologist David Strachan studied the epidemiology of hay fever at the age of 11 and 23 in 17 414 British children born in 1958 (53). At both ages the prevalence of hay fever was inversely related to the number of children in the household. Eczema in the first year of life was also independently related to number of older children in the household. It was suggested that these findings could be explained by infections in early childhood, transmitted by unhygienic contact with older siblings or from the mother (9, 53, 54). This was later known as the so called “hygiene hypothesis”. Many studies followed, but the hygiene hypothesis still remains a hypothesis and the specific underlying mechanism has not been revealed (9, 55-57). Many environmental factors occurring prenatally or during the first years of life have later been associated with the prevalence of allergic diseases (9, 10, 58-61). Traffic-related air pollution in infancy is associated with children’s lung function and sensitization to common allergens (62). Pre- or postnatal exposure to cigarette smoking is a risk factor for development of recurrent wheeze and IgE sensitization to allergens (63, 64). Some factors are considered to be associated with a lower prevalence, like visits to stables and consumption of farm milk or fish during the first year of life (65-67). Several other factors have also been studied in this context, for example exposure to cats and dogs (1), breastfeeding (68), day care attendance (69), farming life (65, 67, 70) and exposure to different types of stress early in life (71). The specific timing of exposure has been studied as well, especially the pre- or early postnatal period (9, 72).

## 2.7 Anthroposophic lifestyle

In the mid-90's focus in our project group was on different possible explanations for the hygiene hypothesis. By personal observations and a conversation to a teacher at a Steiner school in Järna the idea emerged that it seemed like the children in these schools had a lower prevalence of allergic diseases (73). Since the anthroposophic lifestyle included many aspects of life of interest for the hygiene hypotheses the spontaneous idea of a lower prevalence was formulated as a hypothesis. This was the start of extensive research collaboration. The first study by our project group in Steiner schools included 295 children aged 5-13 years at two Steiner schools and 380 children of the same age at two neighbouring schools (12). The children were studied in terms of occurrence of allergic symptoms and laboratory test for allergies. Children from Steiner schools, associated with an anthroposophic way of life, had a lower prevalence of atopy, both by clinical diagnosis and by serological or skin-prick diagnosis (12). This study was followed by a large European multicentre study (11, 13, 74), PARSIFAL, where the findings were confirmed.

The anthroposophic lifestyle is influenced by philosophic ideas founded in the early 20<sup>th</sup> century by the Austrian philosopher and scientist Rudolf Steiner and has a holistic approach (75). These ideas have been applied to many aspects of life, like education (Steiner schools), medicine, art, architecture and agriculture (biodynamic farming). The diet is mainly produced by local farming and produced according to biodynamic principles and includes vegetables preserved by spontaneous fermentation containing live lactobacilli (76). Anthroposophic medicine includes restrictive use of antibiotics, antipyretics and vaccinations and by giving birth at home. For the newborn child it includes specific intentions to reduce stressful environmental stimuli early in life (77). In Sweden the anthroposophic community is centred in the countryside village Järna south of Stockholm.

After the first studies of the influence of anthroposophic lifestyle on the prevalence of allergic diseases in children (12, 78) it became evident that the anthroposophic lifestyle, like every lifestyle, is a very heterogeneous object to study (12). It includes several factors which may hypothetically influence the development of allergic disease and that could be involved in the underlying explanation of the lower prevalence of allergies in school children in Steiner schools (11-13). The previous studies on anthroposophic lifestyle and allergies were of cross sectional design and specific factors responsible for the lower prevalence were not identified. Interesting lifestyle factors within this lifestyle include home delivery, diet and the development of gut micro biota (74, 78), restrictive use of antipyretics (79-81) and antibiotics (82, 83). Further, the intentions to reduce stressful exposure early in life



could also be of interest since psychosocial factors during pregnancy or early in life have been related to allergic diseases (71). The stress-reducing attempts include for example first clothing made by wool or silk, protection of the child from strong light and unpleasant visual stimuli and carefully prepared sensual stimulation (77). To study patterns of exposure in the anthroposophic lifestyle during pregnancy and early in life would possibly give more hypotheses.

## **2.8 Stress and salivary cortisol**

The psychosocial environment including stress early in life may be related to the prevalence of allergic diseases in early childhood (71, 84, 85). Negative psychosocial factors/stress is assumed to be factors with increased prevalence, partly in parallel with the increase in prevalence of allergic diseases (14). Stress is considered to be a reaction in the body to threat and includes both a stressor (the external factor) and the reaction in the body that this causes (86). This reaction includes release of the hormones adrenaline, noradrenalin and cortisol.

Cortisol is the end product of the HPA-axis and has a central role in several organ systems by the regulation of metabolism and stress reactions (87). Sampling of saliva and analyzing techniques were developed in the early 1980s (88). There is a close relationship between serum concentration of free cortisol and concentration of cortisol in saliva (16, 17). Collecting salivary samples for analyses of cortisol is a specific and sensitive method and normative values during infancy have been proposed (17, 89). Studies on cortisol in children includes both analyses of acute reactions to stressor as well as the study of basal levels (90, 91). The impact of environmental factors during infancy on salivary cortisol and the impact of stress on the body have been applied in a large number of studies (92).

The diurnal rhythm of the HPA-axis of children seems to be established during the first months of life with in some studies different reactivity and higher values in younger children (93-95) and the rhythm seems to parallel the development of the sleep-awake cycle (96). Cortisol levels in infants may be influenced by several external factors (95-97). However, feeding type or method did not influence cortisol levels (98). Relational aspects have been studied in relation to HPA-responses, with various results (96, 97, 99-102). Stress responses with altered cortisol levels in children have also been linked to parental experiences (103, 104), to painful medical interventions (105-111) and to trivial daily happenings (97, 112). The development of stress-responses over time seems also to change (113). Child salivary cortisol levels have been correlated to the levels of the mother, regardless of feeding type

(96) but the correlation to levels of the fathers has not been studied before.

## **2.9 Salivary cortisol and allergy**

Psychosocial factors and stress early in life has previously been established as a potential factor influencing the development of many diseases in childhood, including allergic diseases (71, 84, 85). A recent systematic review and meta-analysis exhibited a positive association between psychosocial factors and future atopic disorder as well as between atopic disorders and future poor mental health (14). Pre- and postnatal stress of the mother measured by different stress related questionnaires and lifestyle factors like household income have been related to immunological changes in cord blood and to later development of allergic disease in children (71, 114-119). Postnatal exposure to stress has also been associated with the aetiology of asthma (120, 121). The function of the HPA-axis has been observed to be altered after several types of psychosocial exposures and may also influence immunological processes (122, 123). Increased responsiveness of the HPA-axis to stress has been seen in healthy infants with atopic predisposition and in infants with exposure to risk factors for allergic disease (15, 124, 125). Children with established allergic disease differ in cortisol response to stress and have altered basal cortisol levels compared to healthy children (126-130). Stress and psychological factors have been related to enhanced and prolonged allergic symptoms (131, 132)

## **2.10 Problem formulation**

Studies on the determinants and possible preventive interventions of allergic diseases are needed due to the rising prevalence. Anthroposopic lifestyle has previously been associated to a low prevalence of allergic diseases (11-13). Psychosocial factors have been associated to allergic diseases (14) and levels of cortisol have been noticed as a potential factor related to the development of allergic diseases (15-17). Several questions and hypothesis need clarification:

- I. Previous studies on the anthroposopic lifestyle were cross sectional and performed on school children. There is need for descriptive studies of exposures early in life in families with an anthroposopic lifestyle, to further disentangle specific factors related to the lower prevalence (see Aim I).
- II. A lower prevalence of allergies in school children from families with an anthroposopic lifestyle has been revealed, but whether this effect is apparent already in infancy is still

unknown. The hypothesis is that children from families with an anthroposophic lifestyle have a lower prevalence of allergic sensitization early in life (see Aim II).

- III. Salivary cortisol levels in children have been related to several environmental factors, and also correlated to maternal cortisol levels. Studies on cortisol levels in infancy and the role of environment have to include analyses of cortisol levels in both parents (see Aim III).
- IV. Salivary cortisol is a stress-related hormone that is determined and influenced by factors in the environment. It is not previously studied early in life in relation to anthroposophic lifestyle. The hypothesis is that children from families with an anthroposophic lifestyle have lower levels of salivary cortisol early in life (see Aim IV).
- V. There is an association between allergic diseases and stress/psychosocial factors. It has not known whether an altered HPA-axis early in life, measured by salivary cortisol levels, is associated to the development of allergic diseases. The hypothesis is that higher levels of salivary cortisol early in life are related to an increased prevalence of allergic sensitization and disease (see aim V).

### 3 AIMS

The overall aim of the ALADDIN-study is to study determinants of allergic disease in children. Such factors could be used in the prevention of allergic disease.

This thesis is based on data from the ALADDIN-study. The aim of this thesis was to investigate patterns of lifestyle early in life in relation to salivary cortisol levels in infancy and allergic sensitization and disease during childhood.

The specific aims were:

- I. To describe the study design of the ALADDIN-study and to report on exposure characteristics during pregnancy and infancy of children from families with an anthroposophic lifestyle (**Paper I**).
- II. To investigate the prevalence early in life of IgE-sensitization to common food and inhalant allergens in children from families with an anthroposophic lifestyle compared to other children (**Paper I**).
- III. To investigate the relationships between salivary cortisol levels in six-months-olds and their parents (**Paper II**).
- IV. To investigate if children from families with an anthroposophic lifestyle have lower levels of salivary cortisol that differs from other children (**Paper III**).
- V. To investigate if higher levels of cortisol early in life are associated with an increased prevalence of allergic sensitization and symptoms during the first two years of life (**Paper IV**).

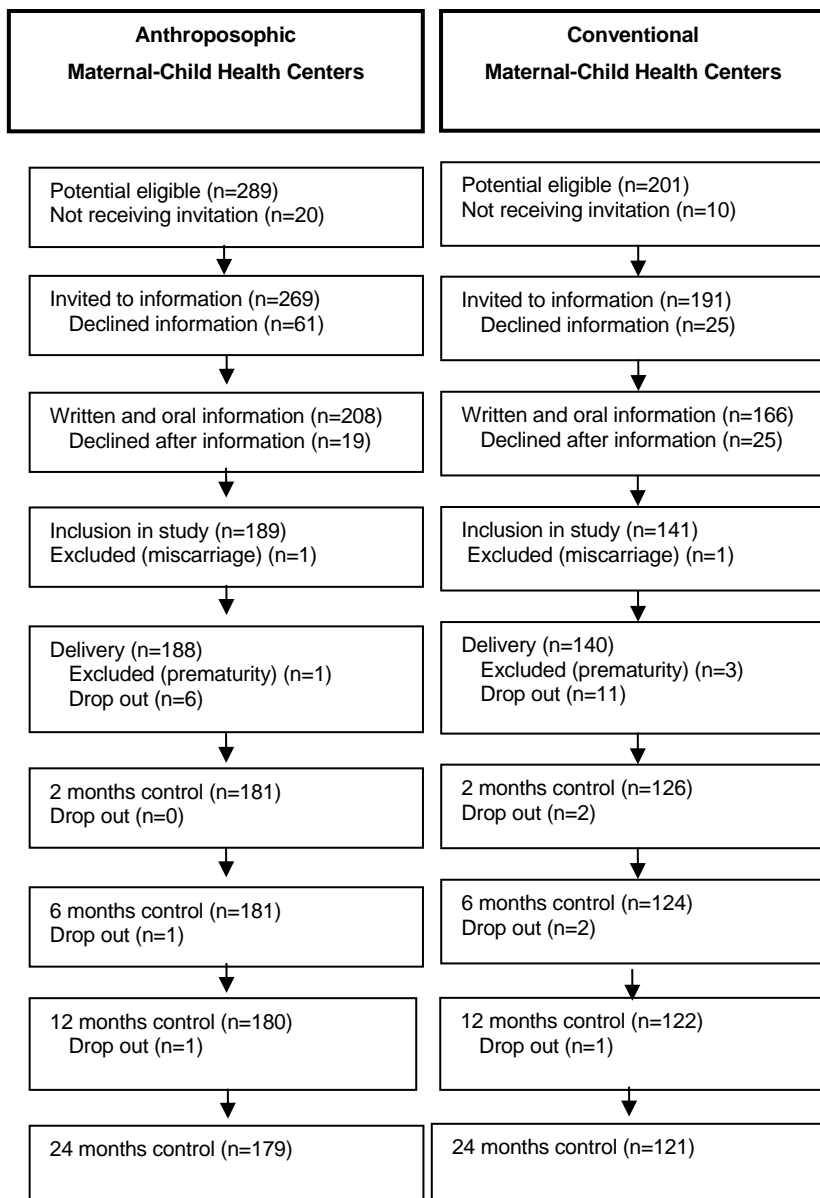
## 4 MATERIAL AND METHODS

The four articles included in this thesis are all based on data from the prospective birth cohort study ALADDIN (Assessment of Lifestyle and Allergic Disease During Infancy), which started in 2004. This Material and Methods section starts with an overview of the whole ALADDIN study followed by descriptions in detail of the specific material and methods used to answer the research questions and aims for this thesis.

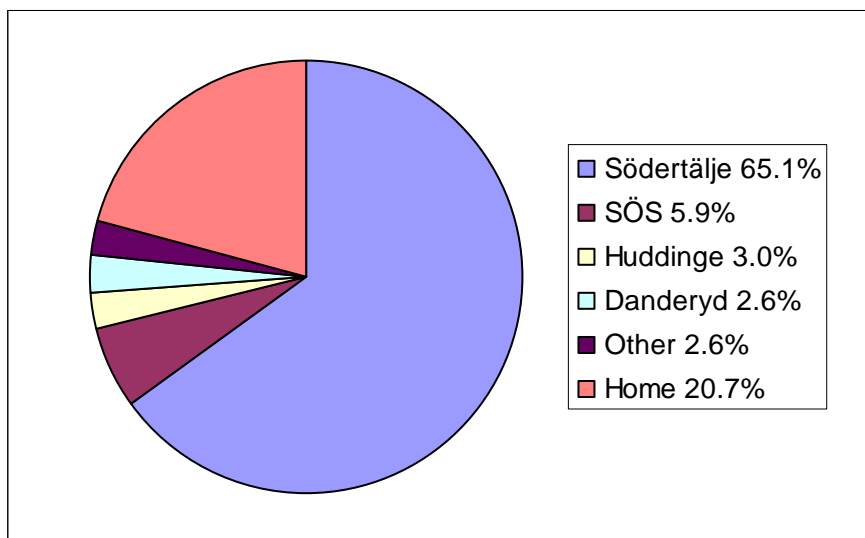
### 4.1 Study population

A total of 330 families were consecutively recruited during pregnancy, between September 2004 and November 2007. Families were recruited from anthroposophic Maternal-Child Health Centers (M-CHC's) in Järna (n = 120) and Stockholm (n = 69) and from conventional M-CHC's in Järna (n = 121) and the nearby town Södertälje (n = 20). The anthroposophic M-CHC's in Järna were Vidarkliniken and Kirstens Familjehälsa and in Stockholm Vidarkliniken at Hälsans Hus and Rosenlund, whereas the conventional MCHC's were Järna Vårdcentral and Oxbackskliniken in Södertälje.

The flow of participants in the study is shown in figure 2. Of all families (n = 201) admitted to non-anthroposophic M-CHC's during the recruitment period all but 10 families got information from their midwife about the study and 25 declined further information. Another 25 of the families received information from the study coordinator but declined participation, leaving 141(70.1%) for inclusion. Corresponding numbers for anthroposophic M-CHC's (n = 289) were 20 families not getting information about the study from their midwife, 61 declining information from the study coordinator, 19 subsequently declining participation and 189 (65.4%) included. The children included in the study were born at delivery clinics in the area or by home birth (figure 3). Infants born before gestational week 36 and miscarriages were excluded from the study. The loss to follow-up is presented in figure 2.



**Figure 2.** Flow chart of the recruitment process and the flow of participants in the ALADDIN-study. (n = number of families at each stage).

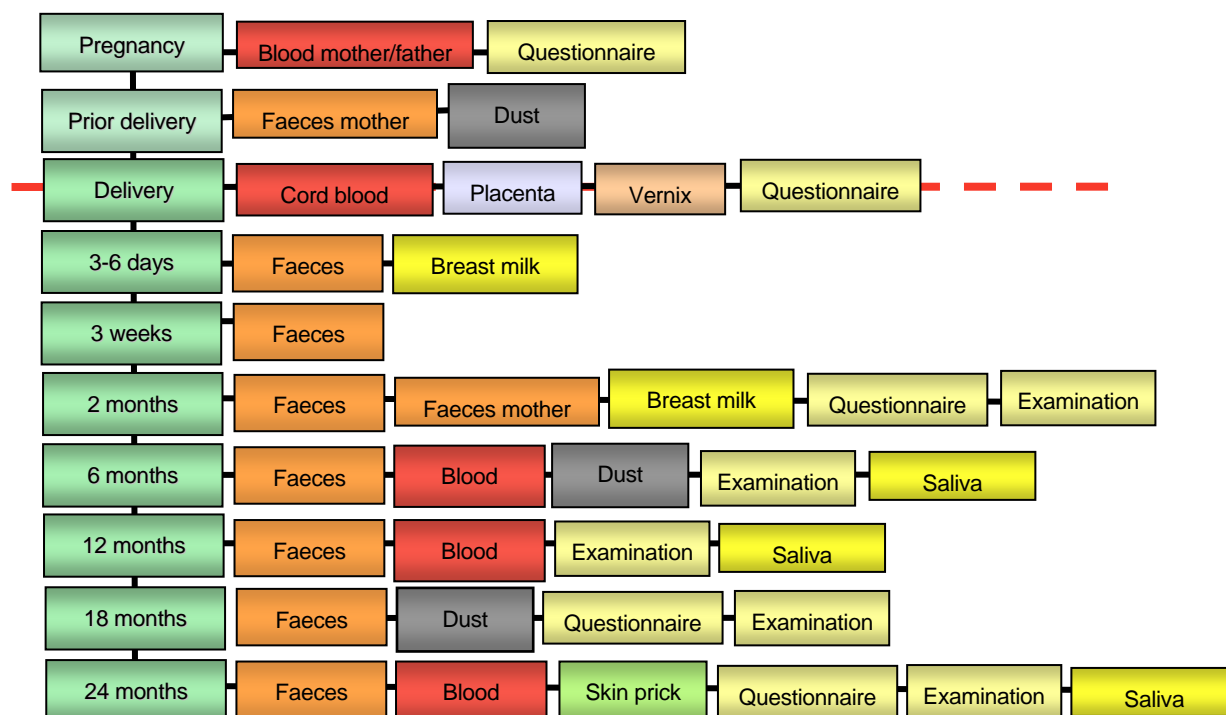


**Figure 3.** Place of birth for the children in the ALADDIN study, hospital or home (SÖS = Södersjukhuset and Södra BB; Danderyd = Danderyd and BB Stockholm; Other = Mälarsjukhuset, Eskilstuna, Nyköpings lasarett, Karolinska Solna).

After study start six families of 330 were excluded: four due to preterm delivery (< 36 gestational week) and two due to miscarriage. Further, before categorization into lifestyle groups (at two months of age) another 17 families did not continue in the study for various reasons: nine due to stressful situations, two due to disease other than allergy of the child or mother, three due to moving, and three for unknown reasons. Five families did not answer the questions used for categorization into lifestyle groups (see below). Thus, after lifestyle categorization the study consisted of 302 families: 82 families with anthroposophic, 120 with partly anthroposophic and 100 with non-anthroposophic lifestyle. After two months of age an additional five families left the study until the age of 24 months: two due to disease other than allergy of the child, one due to moving, and two for unknown reasons.

## 4.2 Study design

The ALADDIN study is a prospective observational birth cohort study where children are followed from pregnancy until the age of two years. In order to analyze different hypothesis concerning the development of allergic diseases during infancy the families are provided to an extensive data collection. An overview of the data collection procedures is presented in figure 4.



**Figure 4.** Flow chart of the data collection procedures in the ALADDIN study.

Demographic and exposure data were obtained from questionnaires and by interviews at the examination occasions. Signs and symptoms of allergic disease were obtained by previously used questionnaires (11, 13, 78), by health diaries, interviews and by physical examinations by the study doctor. Several questionnaires were fulfilled: a questionnaire concerning sense of coherence completed by each parent individually (133, 134), a previously used scale to measure fear during labor and delivery (135) and at 18 months of age a questionnaire on parental social network (136). Specimens from placenta and a sample of vernix from the newborn were collected at delivery (61, 137, 138). Blood samples were collected from the parents during pregnancy, from cord blood and three times from the child during the first two years of life in order to evaluate the allergic sensitization in blood and in coming reports other immunological markers (139-142). Fecal samples were collected from the mother before and after



delivery and from the child at six occasions to further evaluate the relation between lifestyle and gut micro biota in the infant (78, 143). Samples of breast milk were collected twice during the first months of life of the children, in order to evaluate the role of immune competent particles (exosomes) for their role in development of allergies (144). Samples of mattress dust samples were obtained at three occasions in order to evaluate the role of allergens and immune stimulating particles (145, 146). Salivary samples were collected in the morning, afternoon and evening at three occasions (6, 12 and 24 months of age) from the child and both parents. In this thesis the salivary samples from children and parents at six months of age are studied and therefore described in details below.

### **4.3 Salivary cortisol**

At six months of age of the child samples of saliva was collected three times during one day from the child and both parents. For children and parents the sampling occasion in the morning was defined as: “a quarter after awakening and before first meal” and evening as “before going to bed”. The afternoon sampling occasion was for the children defined as “after midday sleep” (or alternatively, if the child did not sleep, “one hour after midday meal”) and for the parents “before dinner or—if dinner was later—before 6 PM”. When analysing the specific time points of sampling it was shown that saliva samples from the same family—parents and infants—were collected almost simultaneously in the morning, but child samples were collected about 2 h before the parents samples in the afternoon as well as at bedtime (see paper III, table 1).

Child saliva samples were collected by sterile rolls (Braided cotton dental rolls; Richmond Dental, Charlotte, NC, USA), which the parents were instructed to keep in the mouth of the child until soaked with saliva. Parental saliva samples were collected by swabs (Salivette; Sarstedt Inc., Rommelsdorf, Germany), which the participants were instructed to keep in their mouth until soaked with saliva. The samples were centrifuged and frozen at  $-70^{\circ}\text{C}$ . All samples were analysed according to the manufacturer’s instructions, using the Spectria Cortisol (125I) kit from Orion Diagnostica, Espoo, Finland (147). Parent and infant samples from the same family were always analysed in the same assay and cortisol levels were expressed in nmol/l. The within- and between assay coefficient of variation never exceeded 5.0 % and 10.0 %, respectively. Comparisons of the two (child and parent) sampling materials were performed by re-analysing 30 adult samples after having re-collected the saliva from the tube, now using the cotton dental roll. The pair-wise correlation between samples thus obtained was 0.995. Previously, a direct comparison of the cortisol analyses in our laboratory has been made with the Kirschbaum laboratory (Technical University of Dresden, Biological Psychology, Professor Dr. Clemens Kirschbaum). Thirty samples were analysed “blindly” in both laboratories. The correlation was 0.98 with systematically

lower levels in the Stockholm laboratory. The difference was 12.5 % with 95% confidence limits 1.5–22.3 %.

#### **4.4 Blood samples**

Blood samples were obtained from both parents at start of the study, from 330/330 of the mothers (100 %) and from 318/330 of the fathers (96.4 %). Blood samples from the children were collected at delivery by aspiration from cord blood, and at the age of six (maximum 5ml), twelve (maximum 5ml) and 24 months (maximum 15ml). The blood sampling was performed by a trained pediatric nurse and in most cases performed using local anesthetic patches (EMLA). The blood samples were collected in heparin tubes and plasma was stored at -20° C. Parental IgE-sensitisation was defined by ImmunoCAP Phadiatop® (Phadia AB, Uppsala, Sweden) containing a mix of 11 inhalant allergens. Cord blood was analyzed for total IgE (Phadia AB). Blood from the children at the age of six, twelve and 24 months was analyzed by ImmunoCAP® (Phadia AB) for total IgE and for IgE to seven common allergens (hen's egg, cow's milk, peanut, cat, dog, birch and timothy).

#### **4.5 Questionnaires and clinical evaluations**

Demographic and exposure data as well as information on signs and symptoms of allergic disease were obtained by previously used questionnaires, health diaries, interviews and by physical examinations (12, 151). The children were followed prospectively from birth until the age of two years and were examined by one of the study doctors at two, six, twelve, 18 and 24 months of age with a particular focus on presence of allergy related disease.

#### **4.6 Lifestyle groups**

The families in the ALADDIN study were recruited from anthroposophic and conventional M-CHC's. To further evaluate the degree of anthroposophic lifestyle a classification of the participating families was performed, based on choice of M-CHC's and parental responses to three questionnaire questions:

1. "What kind of preschool/school will your newborn child probably go to?"
2. "Has any of the parents, no matter which type of school you have planned for your child, an anthroposophic view of life?"
3. "Is the family's everyday life influenced by an anthroposophic view of life?"

Families answering “anthroposophic school” to question 1 and “yes” to question 2 and 3, and also attending anthroposophic M-CHC’s were defined as “anthroposophic”. Families answering conventional or any other non-anthroposophic type of school to question 1, “no” to question 2 and 3, and going to conventional M-CHC’s were defined as “non-anthroposophic”. Any other combination of answers was defined as “partly anthroposophic”.

This classification was done at two months of age when these questions were included in the questionnaire. At this time point 307/330 families still remained in the study (figure 2) and of them five families did not answer the questionnaire, leaving 302 families in the study to be classified into lifestyle groups: 82 were classified as families with an anthroposophic lifestyle, 120 as families with a partly anthroposophic lifestyle and 100 as families with a conventional lifestyle. The lifestyle group classification was used in Paper I, III and IV.

#### **4.7 Sensitization and allergic symptoms**

A parent was classified as IgE-sensitized if IgE levels were  $\geq 0.35$  kU<sub>A</sub>/L in Phadiatop (30). If the Phadiatop test was positive the blood samples was further analysed for the included allergens. A child was classified as IgE-sensitized if IgE levels were  $\geq 0.35$  kU<sub>A</sub>/L for at least one of the seven allergens analysed.

The diagnosis of eczema was clinically assessed by the study doctor and based on the criteria of the UK Working Party’s refinement of the Hanifin and Rajka criteria (148-150). Food allergy was defined as acute onset of symptoms such as skin reactions, wheezing, vomiting, or diarrhea on more than one occasion after ingestion or contact with a particular type of food (12, 151). Recurrent wheeze was defined as three or more episodes of wheeze since the last examination (12, 151).

#### **4.8 Statistical analyses and ethics**

All analyses for Paper I were performed with the statistical software program Statview Graphics II. SPSS version 17.0 was used for all statistical analyses in paper II. Statistical analyses in paper III and IV were conducted using PASW 18.0 software and Stata 11.1. Since saliva cortisol showed a skewed distribution in all the studied groups and on all occasions logarithmic transformation was used in all papers where salivary cortisol was included in the analyses. The log transformed saliva cortisol concentration was normally distributed.

In Paper I the descriptive part of the paper was performed using linear analyses for trend (for categorical variables) and ANOVA (for comparison of

means for continuous variables) for comparison of the prevalence and number of different exposure data between the lifestyle groups. The main comparative analysis, the association between lifestyle groups and sensitization during the first 24 months of age, used generalized estimating equations, which calculates average risk taking the correlation within individuals into account as well as unequally spaced missing observations (152). Median regression was used for total IgE since it showed a skewed distribution. The models in Paper I were adjusted for the following baseline variables: sex of the child, parental sensitization, maternal smoking during pregnancy, number of siblings or other children living with the family, exclusive breastfeeding at six months of age, family living on a farm with animals during pregnancy, and parental education.

The main results in paper II are presented as correlation coefficients with p-values. The analyses were done with one-way ANOVA for correlated means. These analyses gives calculations of statistical significance of variation in saliva cortisol means within the triads (child, mother, father) for each occasion (awakening, afternoon, bedtime) and the corresponding analyses were also performed for the “total slope” (difference between awakening and bedtime concentration).

In Paper III the main statistical comparative analyses was to assess the association between adherence to a specific lifestyle group and levels of salivary cortisol of the children at six months of age. Since the main outcome in these analyses was a continuous variable (salivary cortisol) multiple linear regression models were considered to be the most adequate method (153). The analyses were performed using morning-, afternoon- and evening-values of cortisol of the children as primary outcome in three separate models. To assess the contribution of certain lifestyle factors on the salivary cortisol levels of the infant series of multiple linear regressions were performed with lifestyle group as main exposure factor and cortisol levels as outcome and all exposure factors that significantly differed between the groups as covariates.

The main statistical analyses in Paper IV were to assess the association between salivary cortisol at six months of age of the child in the morning, afternoon and evening in relation to sensitization, eczema, food allergy and recurrent wheeze during the first two years of life. General estimated equations were used for these analyses. The models in Paper IV were adjusted for sex of the child, lifestyle group, parental sensitization, mother smoking during pregnancy, number of siblings or other children living with the family, exclusive breastfeeding at six months of age, family living on a farm with animals during pregnancy and parental education.

The study was approved by the Research Ethical Committee at Huddinge University Hospital, Stockholm, Sweden. Written informed consent was obtained from all families.

## 5 RESULTS

### 5.1 Exposure characteristics in the lifestyle groups

Anthroposophic lifestyle has previously been described to be protective to allergic diseases, although only shown in cross-sectional studies and in older children (11-13). Many specific lifestyle factors in this lifestyle seem to be interesting in this context, especially prenatally and early in life. Therefore there is need for an intensive study description of specific lifestyle factors in families with an anthroposophic lifestyle during pregnancy, delivery and early life. These factors will later be analysed specific in relation to the development of allergic diseases.

Differences and similarities between anthroposophic and conventional lifestyle groups were seen in demographic as well as pre- and postnatal environmental factors. The parents in the three groups did not differ in age at birth of the child. The majority of parents in all three lifestyle groups were born in Scandinavia. However, in the anthroposophic group parents to a higher extent were born abroad, mostly in another European country. The mothers born in non-Scandinavian European countries were born in Germany (14/39, 35.9 %), Finland (9/39, 23.1 %), Poland (4/39, 10.3 %) and other countries (12/39, 30.7 %). Similar data for the fathers were Germany (16/44, 36.4 %), England (7/44, 15.9 %), Finland 4/44 (9.1 %), Austria 4/44(9.1 %) and other countries (13/44, 29.5 %). The education level of the parents was similar between the three groups concerning studies at university, but the mothers in anthroposophic groups had a lower degree of studies at gymnasium. Families with non-anthroposophic lifestyle more often lived in private houses as compared to the other groups, whereas the families with anthroposophic lifestyle more often lived in apartments and had condensation on the windows. Having older siblings did not differ between the groups, but number of older siblings was significantly higher in the anthroposophic group.

Reported symptoms of allergy (eczema, asthma, allergic rhinoconjunctivitis and reactions to food) in the parents or siblings did not differ significantly between the three lifestyle groups. Parental allergic sensitization did not differ between the groups. In total 27.2 % of the mothers were IgE-sensitized and 43.0 % of the fathers.

Several factors differed between the groups during pregnancy and delivery. In the anthroposophic group 41.5 % of the children were born with home delivery, compared to 24.2 % of the partly-anthroposophic and none in families with non-anthroposophic lifestyle. The number of children born with caesarean section did not differ between the groups. Mothers from the anthroposophic group did to a lower degree get anesthetics during delivery and had often a midwife from M-CHS's present at delivery. Birth height, length and head circumference did not differ between the groups. Children in the non-anthroposophic were given cow's milk formula during the first

week more often than other. A majority of the children from the anthroposophic families had their first clothing made by wool, compared to the non-anthroposophic group where this was not present at all. The use of antipyretics and antibiotics did not differ between the groups during the first two months of age. Children from the anthroposophic group received nature-cure medicine more often.

At two months of age children in anthroposophic families were to a higher extent exclusively breastfed as compared to children in the other groups. Maternal diet during breastfeeding differed significantly. Mothers in the anthroposophic group to a higher degree consumed an organic and/or biodynamic diet and used more olive oil and butter in food and more often a vegetarian diet. Further, children in anthroposophic families were less often given AD-vitamin supplement, and more commonly wore wool closest to the skin and lived on a farm with animals.

## **5.2 The association between lifestyle group and sensitization**

Sensitization was most prevalent in the children from the families in the non-anthroposophic group at each follow up – six, twelve and 24 months of age (table 1). Children from families with an anthroposophic lifestyle had an overall decreased risk of sensitization during the first two years of life compared with children from non-anthroposophic families with an OR of 0.25 (95 % CI 0.10-0.64), p-value 0.004. Children from families with a partly anthroposophic lifestyle also showed a decreased risk compared with children from the non-anthroposophic families, adjusted OR 0.31 (95 % CI 0.15-0.54), p-value 0.002 and did not differ significantly compared to children from anthroposophic families. The level of total IgE in cord blood and during the first two years of life did not differ significantly between the three lifestyle groups.

	<b>Anthro.</b>		<b>Partly anthro.</b>		<b>Non-anthro.</b>	
<b>Cord blood</b>						
<b>Total IgE</b>	<b>0.32</b>		<b>0.16</b>		<b>0.18</b>	
<b>6 months</b>						
<b>Sensitization</b>	<b>1/51</b>	<b>(2.0)</b>	<b>6/84</b>	<b>(7.1)</b>	<b>16/82</b>	<b>(19.5)</b>
Egg	1/56	(1.8)	3/97	(3.1)	6/86	(7.0)
Milk	0/57		0/97		10/87	(11.5)
Peanut	0/56		1/96	(1.0)	0/86	
Cat	0/52		2/88	(2.3)	1/82	(1.2)
Dog	0/51		0/84		2/82	(2.4)
Timothy	0/53		0/87		0/83	
Birch	0/54		0/86		0/83	
<b>Total IgE</b>	<b>3.6</b>		<b>4.0</b>		<b>5.2</b>	
<b>12 months</b>						
<b>Sensitization</b>	<b>2/47</b>	<b>(4.3)</b>	<b>10/87</b>	<b>(11.5)</b>	<b>26/83</b>	<b>(31.3)</b>
Egg	2/53	(3.8)	5/92	(5.4)	16/91	(17.6)
Milk	0/53		3/91	(3.3)	12/91	(13.2)
Peanut	0/51		2/90	(2.2)	4/89	(4.5)
Cat	0/50		1/88	(1.1)	4/81	(4.9)
Dog	0/46		0/87		3/81	(3.7)
Timothy	0/50		0/86		1/81	(1.2)
Birch	0/51		0/88		1/85	(1.2)
<b>Total IgE</b>	<b>6.6</b>		<b>6.8</b>		<b>7.3</b>	
<b>24 months</b>						
<b>Sensitization</b>	<b>9/59</b>	<b>(15.3)</b>	<b>11/89</b>	<b>(12.4)</b>	<b>24/90</b>	<b>(26.7)</b>
Egg	1/59	(1.7)	3/92	(3.3)	13/92	(14.1)
Milk	6/59	(10.2)	7/93	(7.5)	13/92	(14.1)
Peanut	0/59		4/92	(4.3)	4/92	(4.3)
Cat	1/59	(1.7)	0/92		4/91	(4.4)
Dog	0/59		0/90		4/90	(4.4)
Timothy	0/59		2/89	(2.2)	5/91	(5.5)
Birch	2/59	(3.4)	4/90	(4.4)	4/91	(4.4)
<b>Total IgE</b>	<b>15.0</b>		<b>15.0</b>		<b>14.5</b>	
<b>OR*</b>	<b>0.25 (0.10-0.64)</b>		<b>0.31 (0.15-0.54)</b>		<b>1.0 (reference)</b>	
p-value		0.004		0.002		-
<b>OR<sup>#</sup></b>	<b>1.0 (reference)</b>		<b>1.24 (0.46-3.33)</b>		<b>4.03 (1.57-10.4)</b>	
p-value		-		0.66		0.004

**Table 1.** Total IgE in cord blood, and sensitization to common allergens and total IgE at six, twelve and 24 months of age in children of families with an anthroposophic, partly anthroposophic and non-anthroposophic lifestyle. (Sensitization n/N (%). Total IgE: median kU/L).

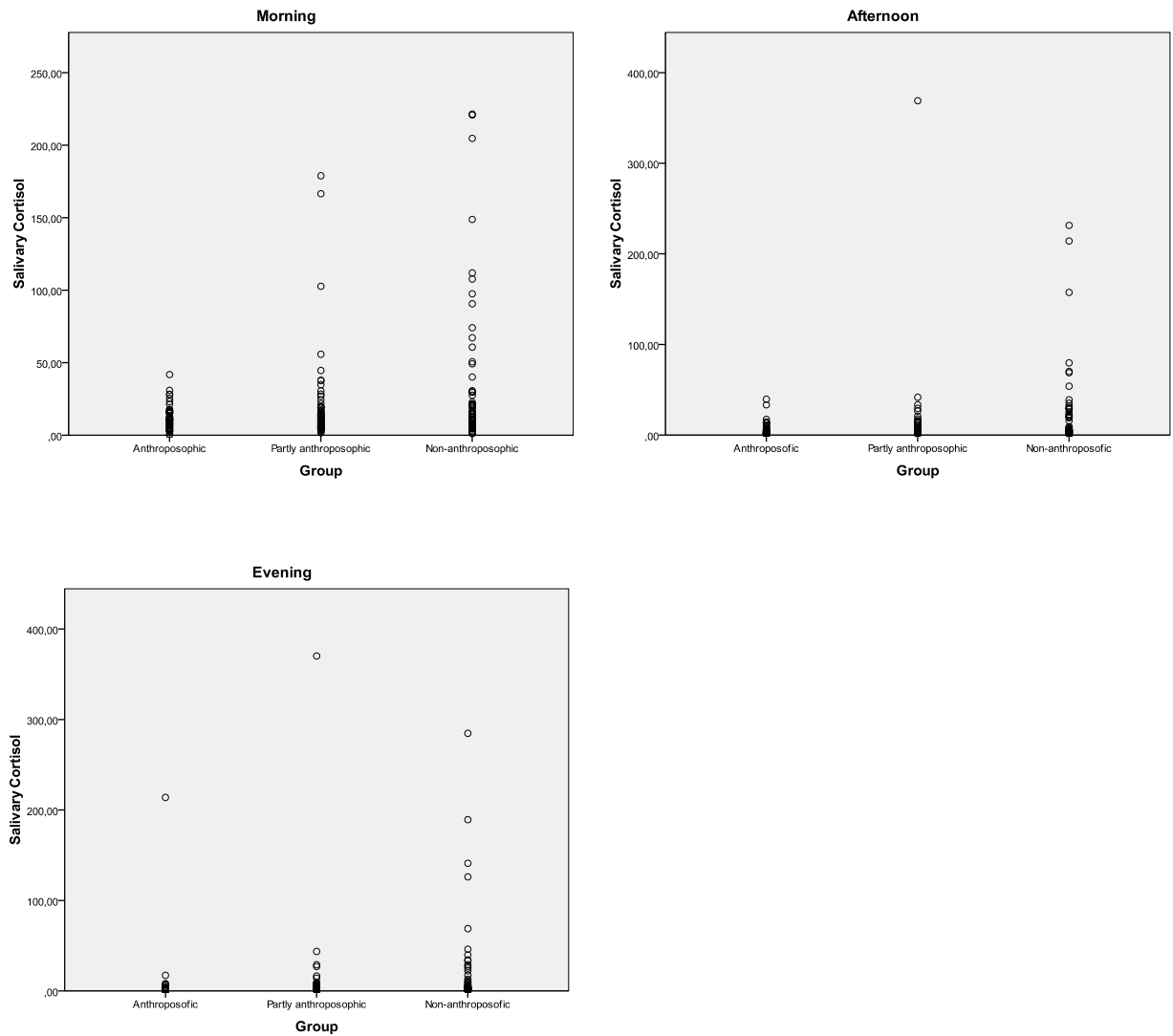
### **5.3 The relationships between salivary cortisol in six-months-olds and their parents.**

Comparisons were made between the child and each parent separately at the three sampling times during the day and also between the slope of the curve (difference between morning and bedtime saliva cortisol). Salivary cortisol in the morning from the mother and from the child showed a strong relationship. The afternoon mother–child levels were also strongly related to one another and there was also a correlation between father–child although it was weaker than the mother-child relationship. In the evening, the mother–child relationship was again strong and the father–child relationship slightly weaker. Correlation analysis showed that there was a strong association between the mother’s and the child’s slope. The association between the father’s and the child’s slope was also significant, but less convincing. As a control, similar comparisons (differences between morning and bedtime saliva cortisol) were performed using data from the children and non related parents. No significant correlations were established in these analyses.

### **5.4 The association between lifestyle and salivary cortisol levels**

Infants of families with an anthroposophic lifestyle had significantly lower cortisol levels on all three sampling occasions compared to infants in the partly anthroposophic and non-anthroposophic groups. In particular, there seemed to be a lack of infants with high salivary cortisol levels in the anthroposophic group (figure 5). For example the morning value of salivary cortisol was 8.8 nmol/l (6.7 – 11.5) in the anthroposophic group, compared to 11.3 (9.3 – 13.7) in the partly anthroposophic group and 14.9 (11.3 – 19.6) in the non-anthroposophic group ( $p = 0.018$ , geometric means with 95 % CI). The parental cortisol levels on the three sampling occasions did not differ significantly between the three groups, either in the mothers or fathers. There were no differences between the groups in their answers to the short questionnaire regarding unusual events and health conditions during the day of saliva collection.



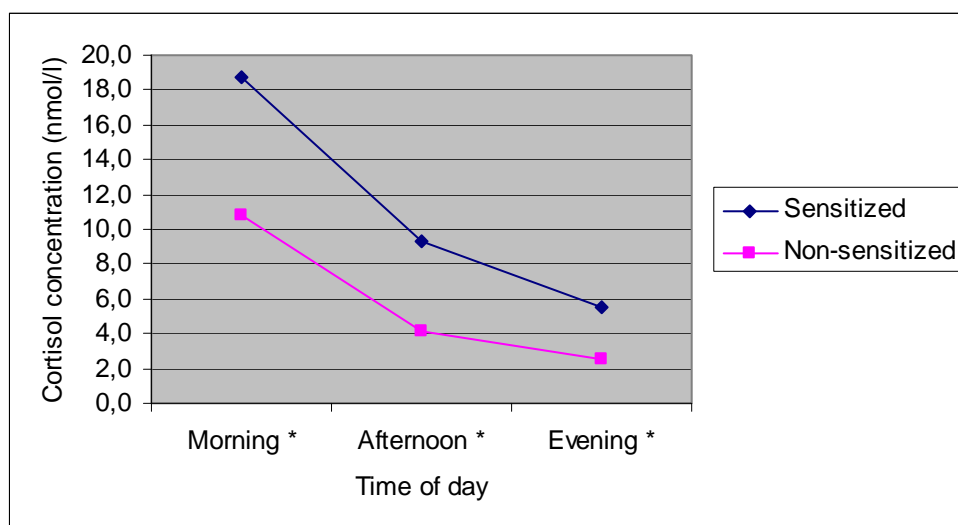


**Figure 5.** Salivary cortisol levels (nmol/l) in the children in the three lifestyle groups at six months of age on the three sampling occasions.

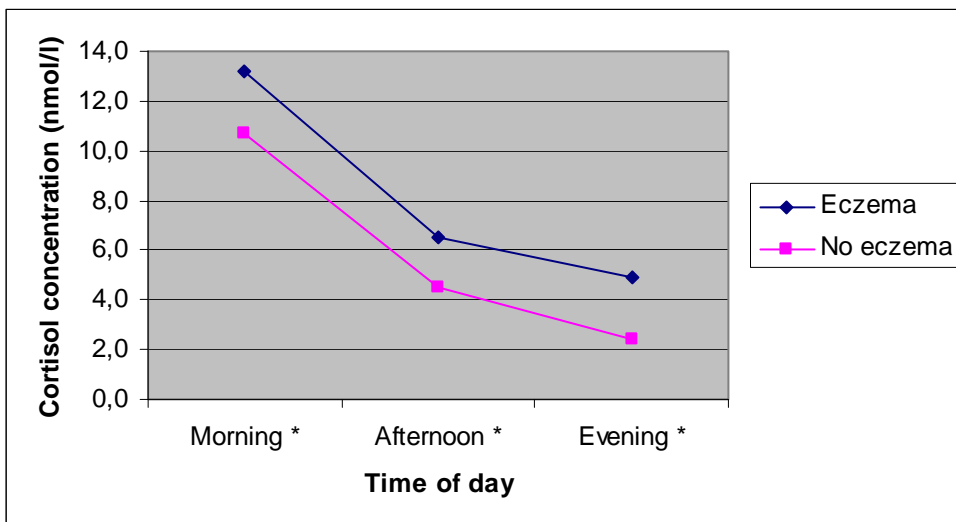
The contribution of specific lifestyle factors that differed significantly in prevalence between the groups on cortisol levels were analysed with multiple models. The independent contribution of the lifestyle group factor on the cortisol levels in the morning, afternoon and evening of the infant was confirmed in all analyses. None of the specific exposure factors analysed could independently explain the lifestyle correlation. One factor, mother living on a farm during pregnancy, contributed significantly in the multiple regression analysis to morning cortisol levels of the infants, but this was independent of lifestyle group. Thus, in multiple linear regression analysis both lifestyle group and mother living on a farm had independent significant effects on salivary cortisol with p-values of 0.010 and 0.019, respectively.

## 5.5 Salivary cortisol in relation to allergy

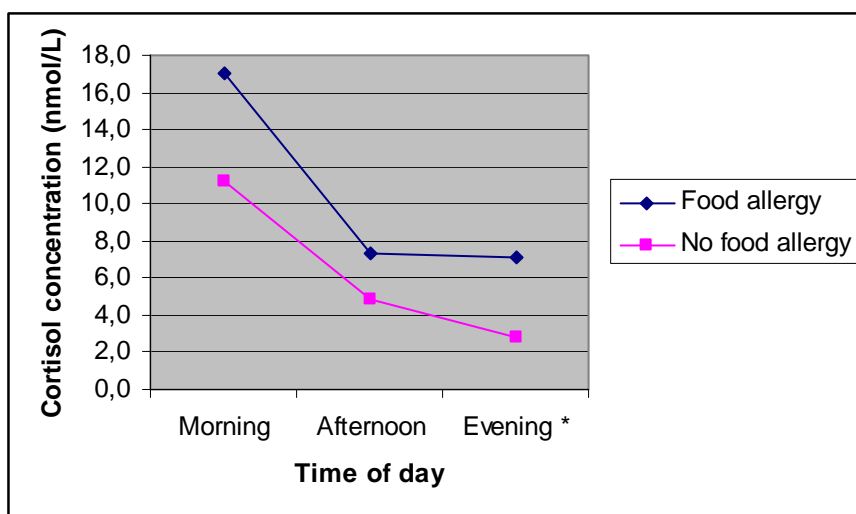
The levels of salivary cortisol in children at six months of age were measured at three occasions during one day. These levels were separately analysed in relation to the prevalence of different allergic manifestations during the first two years of life: sensitization, eczema, food allergy and recurrent wheeze. Salivary cortisol levels were on all sampling occasions related to risk of sensitization and eczema during the first two years of life, with increasing values of cortisol giving higher risk (figure 6 and 7). The overall adjusted OR for the association between sensitization and the morning value of salivary cortisol was 1.60 (95 % CI 1.22-2.10,  $p = 0.001$ ), for afternoon values of salivary cortisol 1.56 (95 % CI 1.26-1.94,  $p < 0.001$ ) and for the evening value 1.49 (95 % CI 1.22-1.83,  $p < 0.001$ ). The corresponding adjusted OR for eczema in relation to morning cortisol was 1.28 (95 % CI 1.03-1.59,  $p = 0.026$ ), afternoon 1.32 (95 % CI 1.12-1.58,  $p = 0.001$ ) and evening 1.37 (95 % CI 1.18-1.59,  $p < 0.001$ ). Salivary cortisol in the evening was significantly correlated to subsequently reported food allergy with overall OR 1.29 (95 % CI 1.04-1.61,  $p = 0.022$ ) but morning and afternoon cortisol was not related to food allergy (figure 8). Salivary cortisol was, however, not related to recurrent wheeze at any time point, but there was a tendency to decreased risk with higher values of cortisol (figure 9).



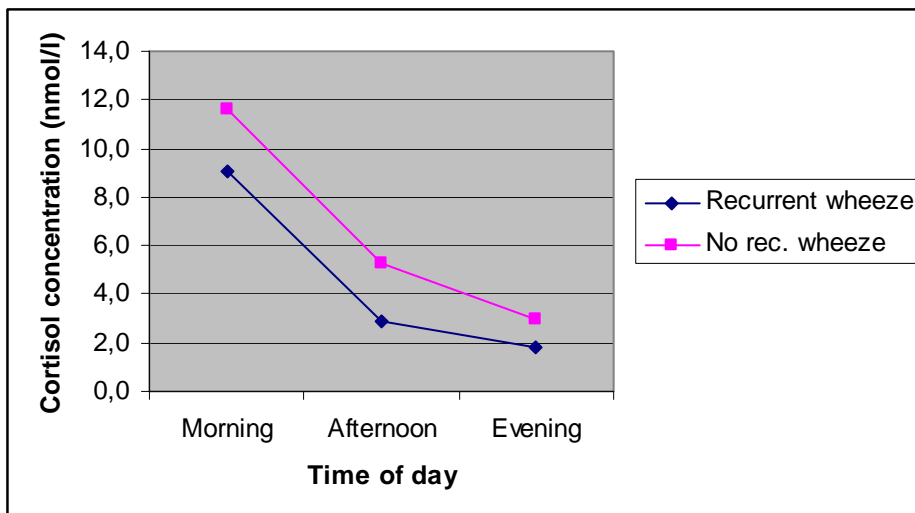
**Figure 6.** Cortisol concentration in children at six months of age (geometric means) at different time-points during the day in sensitized and non-sensitized children during the first two years of life. \*  $p \leq 0.05$ .



**Figure 7.** Cortisol concentration in children at six months of age (geometric means) at different time-points during the day in children with eczema or not during the first two years of life. \*  $p \leq 0.05$ .



**Figure 8.** Cortisol concentration in children at six months of age (geometric means) at different time-points during the day in children with food-allergy or not during the first two years of life. \*  $p \leq 0.05$ .



**Figure 9.** Cortisol concentration in children at six months of age (geometric means) at different time-points during the day in children with recurrent wheeze or not during the first two years of life.

For the two outcomes that were significantly associated to cortisol on all three occasions (sensitization and eczema) analyses were also done restricted to healthy children (not sensitized or without eczema) at six months of age. This was done in an attempt to analyze the impact of cortisol levels at six months of age on only later development of sensitization and eczema (12-24 months of age). In children without sensitization at six months of age there was a close to significant association between salivary cortisol in the afternoon at six months of age and sensitization from twelve to 24 months of age, with adjusted OR of 1.43 (95 %CI 1.00-2.06,  $p = 0.051$ ). The corresponding OR's for the morning and evening value was 1.30 (0.90-1.87,  $p = 0.16$ ) and 1.29 (95 % CI 0.95-1.75,  $p = 0.11$ ). Salivary cortisol at six months of age following this exclusion was not related to development of eczema between twelve and 24 months of age.

## 6 DISCUSSION

### 6.1 Findings

#### *6.1.1 Exposure characteristics in the three lifestyle groups*

Several life style factors early in life were associated with the anthroposophic lifestyle, some of them previously assessed in relation to development of allergic diseases, such as family size, diet and living on a farm (9, 10, 53, 54), but some of them not studied before. Previous studies of lifestyle factors in the anthroposophic lifestyle (11-13) were performed in older children, and no previous study have to our knowledge made such a thorough and detailed characterization of this lifestyle during pregnancy, delivery and postnatal period of life. Although so far not further studied in detail in relation to further development of allergic diseases and in relation to the massive biological sampling, this characterization in the ALADDIN study may give insights and hypothesis for coming papers in the study.

#### *6.1.2 The association between lifestyle group and sensitization*

The low risk of IgE mediated sensitization in the children in families with an anthroposophic lifestyle is in accordance with earlier results (11-13). However, these studies were cross sectional and performed in schoolchildren and the present results indicate an early effect on allergic diseases not shown before. When comparing the different time points (6, 12 and 24 months of age) it was actually seen that the effect of lifestyle was strongest at the first time points (6 and 12 months). This may indicate that an anthroposophic lifestyle has an early strong effect or that it postpones the debut of allergic symptoms. The findings is in line with other cohorts where exposure early in life to tobacco smoke, pollution from traffic and exposure to farming animals during fetal period have been related to allergy development (62-65). The prevalence of allergies in the non-anthroposophic group was similar to other studies (154, 155). The results on a difference in sensitization are largely explained by the difference in proportion of IgE-sensitization to food allergens, which constitutes the most common type of sensitization during the first year of life (32). Anthroposophic parents were born to a higher degree in non-Scandinavian European countries, but their allergy prevalence and sensitization did not differ. Thus, it seems unlikely that genetic factors could explain the lower prevalence of sensitization in children in the anthroposophic group.

### *6.1.3 The relationships between salivary cortisol in six-months-olds and their parents.*

Correlations were found between salivary cortisol levels in six-months-olds and their parents, for the mother on all three sampling occasions and for the father only in the afternoon and evening. The correlations were stronger for mother-child correlation. This may indicate environmental influence of the caregiver on the child. Genetic influence on child cortisol levels is often considered to be strongest on the morning value of cortisol (156-158), which may be surprising since we found no correlation between father-child in the morning, but contrasting results concerning this has been shown before (156, 159). The findings of no correlation between breast feeding and cortisol levels, studied before in different contexts (96, 160, 161) may be due to the low number of bottle fed infants.

### *6.1.4 The association between lifestyle and salivary cortisol levels*

Our findings of lower levels of salivary cortisol in infants, but not in parents, from families with an anthroposophic lifestyle may reflect that early infancy is a period when certain stimuli may evoke cortisol reactions while such reactions will diminish or disappear over time (113). The saliva cortisol levels in the parents were within normal levels compared with other studies (162). Our findings could also suggest that potentially stress reducing environmental conditions related to the anthroposophic lifestyle (77) may be more relevant for infants than for adults. Considerable efforts were made to find possible explanations for the findings in the environmental factors that differed between the groups. Surprisingly, beside the lifestyle group factor, only one factor, mother living on a farm during pregnancy, was significantly associated with salivary cortisol levels in the children. Prenatal exposure to farming has previously been associated with a lower risk of developing allergic diseases (65, 67, 70). However, this factor did not explain the association between anthroposophic group and salivary cortisol concentration in the multivariate analysis. One hypothesis is that anthroposophic families provide an infancy environment with a lower degree of exposure to every day stressors (77), but more detailed data on this from questionnaires in the ALADDIN study is limited.

The value and meaning of low cortisol levels have been studied in other settings, not only in relation to allergy and anthroposophic lifestyle (163). Children with anthroposophic lifestyle have lower levels of cortisol in our study, but the implication for this into adulthood is not known. We are not aware of any published long-term follow-up studies of the health during adult years in infants who were brought up in anthroposophic families. However, one study has shown a lower prevalence of hypertension and degenerative arthrosis during adulthood in former Steiner school children (164). Still, any conclusions about health effects related to the low cortisol levels in the anthroposophic children should be regarded with caution.

### *6.1.5 Salivary Cortisol in relation to Allergy*

The associations between salivary cortisol in infancy and allergic sensitization and eczema during the first two years of life indicate a role for an altered HPA-axis in the development of allergic diseases. This has previously been studied (71, 114-121, 124, 125), but not to our knowledge analyzed by measuring cortisol levels in early life and following children prospectively. A blunted cortisol response to stress (hyporeactivity) and lower basal levels of cortisol has been seen in children with established allergic disease (126, 127). Our results with higher levels early in life associated to allergic manifestations may be a sign of hyperreactivity before or at the onset of allergic disease (128).

The statistical methods used show strong associations, but the direction of the relation has to be interpreted with care. The results follow the original hypothesis, which was stated prior to study start. Children with established eczema could be regarded as stressed when observed in the clinical settings and could therefore be suspected to have raised levels of the stress hormone cortisol. However, many studies on children with already developed eczema and allergic disease show lower or blunted levels of cortisol (126, 127).

The etiology of allergic diseases is complex and crucial mechanisms remain to be explained. A change in environmental and lifestyle factors is probably involved in the rapid changes in prevalence of allergic diseases, of which those occurring prenatally or during the first year of life may be most influential (9). Our results indicate a relation early in life between cortisol and risk for allergies. One could argue that there is weak evidence that stress in infancy has increased and thus not fulfill criteria for being engaged in the raised prevalence of allergic diseases. However, other factors early in life influencing the development of allergic diseases, besides stress, may do this directly or indirectly by influencing the HPA-axis. In addition, a “stress-modified” immune system could be more susceptible to environmental influences, including such associated with risk for allergy. Consequently, infants in a low-stress environment would to some extent be protected to an allergic immune response even if exposed to a similar environment. The influence on the HPA-axis of the child from the mother prenatally or during the postnatal period are considered in many studies, not only involving allergic diseases. The two major signaling systems of the body – the endocrine system and the immune system – are in many ways connected and signaling between these systems plays a crucial role in the body (165). The results in our study could indicate that lifestyle may influence the relation between these two systems during early childhood.

## **6.2 Methodological aspects**

### *6.2.1 Study design*

The ALADDIN study was designed as a prospective observational birth cohort study. Previous studies on the influence of anthroposophic lifestyle on allergic diseases in children were all of cross-sectional design and specific factors of the lifestyle were difficult to disentangle. Epidemiologic studies can be seen as exercises undertaken to obtain estimates of epidemiologic measures (166). There are two main types of epidemiologic studies, the cohort study and the case-control study (166). Several factors are involved in the choice of study design. When studying an exposure that is rare and a disease that is more common the cohort study is to be preferred. Therefore for the research questions in this thesis the choice was made to use the cohort design and the ALADDIN study was initiated, since the study evaluate an uncommon exposure (anthroposophic lifestyle) with an common outcome (allergic diseases). A cohort can principally be observational or experimental, where the observational study follows the study population without interventions. An experimental cohort study is for example the randomized clinical trial. Since it is not possible to randomize parents into choice of lifestyle during pregnancy and early life of their child the natural choice was to perform a prospective observational cohort study. The families were recruited from both anthroposophic and conventional health care centres in order to achieve families with different lifestyles and different exposure to environmental factors. To further evaluate the role of anthroposophic lifestyle families were classified into lifestyle groups based both on choice of M-CHS's and answer to questionnaires.

### *6.2.2 Systematic and random errors*

Two broad types of error can affect epidemiologic studies: random error and systematic error (166). Another term for systematic errors is bias. A study can be biased because of the way the study population was selected and followed (selection bias), how the different variables were measured (information bias) and because of some other factor influencing the results and not completely controlled for (confounding). The two populations in the study received information and were recruited by the same procedure, minimizing risk for selection bias. The recruitment rate was fairly high (65-70% at both anthroposophic and conventional M-CHC's) indicating that the cohort could be representative for the populations targeted for investigation. The loss to follow-up was very small in all lifestyle groups, minimizing risk of such bias.

Information on exposure characteristic for an anthroposophic lifestyle was collected by questionnaires, health diaries and interviews, which may lead to information bias. This problem is however more evident in a retrospective cohort study and in case-control studies. In these study designs the problem of recall bias can occur. To prevent this bias was one of the reasons to choose the prospective design of the ALADDIN study. The



base-line information was received already during pregnancy and neonatal period and thus before allergy related symptoms were expressed or test results available. This should minimize risks of disease related modification of exposure, meaning that parental answers to questions on different exposure could be related to the development of disease in their children. This type of bias may also be more evident in a case-control study.

Blood samples were available in more than 80 % of the children from at least one time point. Some part of the missing blood values was explained by failure by the nurse to receive blood, and this was equally distributed between the groups. The choice of not giving blood was made by the parents at a time point when they had not received any results of their own or the child's blood sample analyses, so it seems unlikely that their choice of not giving blood was affected by allergic disease. All families received their results of the blood samples from parents and children after the last child in the study had turned 24 months of age. The choice of not giving blood may be more related to lifestyle associated attitudes. The variables cortisol and sensitization were based on objective measurements independent of each other. To avoid influence on cortisol levels of blood sampling procedures, collection of salivary samples was scheduled to not interfere with the health care visit and blood drawing. The study doctor was blinded to salivary cortisol levels and blood sample results when doing clinical assessments.

Many factors in the anthroposophic lifestyle could be explanatory factors for the results of the lower prevalence of allergic sensitization and lower levels of salivary cortisol. In paper III we assessed this by analysing the models for all lifestyle factors that differed significantly between the groups and found no explanatory factor. Still it is possible that some other unknown factors are associated to the findings. Cortison treatment in children should be taken into account when analyzing salivary cortisol levels, since this treatment may influence levels of salivary cortisol (17, 126, 167), and could potentially be a confounding factor. No family reported any treatment with oral or inhaled corticosteroids at six months of age of the children when salivary cortisol was analysed. The associations between salivary cortisol levels, sensitization and allergic symptoms were adjusted for lifestyle group (anthroposophic, partly anthroposophic and non-anthroposophic). This is attempted to reduce the influences of confounding due to previously reported characteristics of children from families with an anthroposophic lifestyle.

## 7 CONCLUSIONS

The following conclusions can be drawn from the presented papers:

- I.** Many exposure characteristics before birth and early in life differ between children from families with an anthroposophic lifestyle in comparison to other children. These include for example diet, home deliveries, medication, household size and number of older siblings.
- II.** The prevalence of IgE-sensitization to common food and inhalant allergens in children is lower among children from families with an anthroposophic lifestyle compared to other children during the first two years of life.
- III.** Correlations were found between salivary cortisol levels in six-months-olds and their parents, for the mother on all three sampling occasions and for the father in the afternoon and evening.
- IV.** Children from families with an anthroposophic lifestyle have lower levels of salivary cortisol at six months of age.
- V.** High levels of salivary cortisol at six months of age are associated to an increased prevalence of IgE-mediated sensitization and eczema during the first two years of life.

## 8 SVENSK SAMMANFATTNING

### Bakgrund

IgE-medierade allergiska sjukdomar (eksem, födoämnesallergi, astma och rinokonjunktivit) är bland de vanligaste kroniska sjukdomarna under barndomen. Förekomsten har ökat framför allt i västvärlden under de senaste decennierna, även om en viss stabilisering kan ses i områden med hög prevalens. I Sverige drabbas 30-40 % av barnen av någon form av allergisk manifestation under sin uppväxt. Förståelsen av allergiska sjukdomar hos barn och det förebyggande arbetet kring dessa förutsätter fortsatta studier av bakomliggande faktorer. Olika miljö- och livsstilsfaktorer under graviditet och tidig barndom tros ligga bakom den snabba ökningen av allergiska sjukdomar. Den antroposofiska livsstilen har tidigare visats associerad till en lägre prevalens av allergier hos skolbarn. Psykosociala faktorer liksom stresshormonet cortisol har satts i samband med allergisk sjukdom.

### Syfte

Det övergripande syftet med denna avhandling var att undersöka samband mellan livsstil under graviditet och tidigt i livet i förhållande till salivkortisol och utvecklingen allergisk sensibilisering och allergiska symptom hos barn.

### Metod

Materialet i denna avhandling baserar sig på ALADDIN-studien, en prospektiv födelsekohort studie, där 330 familjer rekryterades under graviditeten. Studien startade 2004. Familjerna delades in i livsstilsgrupper med olika grad av antroposofisk livsstil, baserat på enkätfrågor och val av MVC/BVC. Data om miljö- och livsstilsfaktorer samlades in via enkäter och intervjuer. Barnen undersöktes vid upprepade läkarundersökningar med avseende på allergiska symtom. Salivprover för analys av kortisol samlades in vid tre tillfällen under en dag vid sex månaders ålder från både barn och föräldrar. Blodprover för analys av allergiantikroppar samlades in från föräldrarna under graviditeten och från barnet vid födseln samt vid sex, tolv och 24 månaders ålder.

### Resultat

Det första arbetet i avhandlingen visar att många livsstilsfaktorer under graviditet och tidig barndom skilde sig hos barn från familjer med antroposofisk livsstil jämfört med andra barn i studien. Dessa faktorer inkluderade t ex kost, hemförlossning, medicinering och hushållsstorlek. IgE-sensibilisering mot vanligt förekommande allergen var under de två första levnadsåren signifikant lägre hos barn från familjer med antroposofisk livsstil. I det andra arbetet påvisas samband mellan salivkortisol hos barn vid sex månaders ålder och båda deras föräldrar. Sambanden var starkare vid alla tidpunkter mellan mamma och barn än mellan pappa och barn. Det tredje arbetet visar att barn från familjer med antroposofisk livsstil hade lägre nivåer av salivkortisol vid sex månaders

ålder, både på morgon, eftermiddag och kväll. I det fjärde arbetet visas att högre nivåer av salivkortisol vid sex månaders ålder var associerat till en ökad förekomst av allergisk sensibilisering och eksem under de två första levnadsåren, detta oavsett vilken av livsstilsgrupperna i studien familjerna tillhörde.

### **Konklusion**

Avhandlingen påvisar associationer tidigt i livet mellan livsstil, salivkortisol och allergi. Dessa resultat tillför ny kunskap kring mekanismerna kring allergisk sjukdom hos barn, vilket förhoppningsvis kan komma att vara en del av framtida preventiva åtgärder.

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