

Association of Apolipoproteins with Symptoms of Asthma and Atopy among Schoolchildren

Gabriele Nagel^a Stephan K. Weiland^{a, 1} Kilian Rapp^a Bernhard Link^c
Iris Zoellner^c Wolfgang Koenig^b

^aInstitute of Epidemiology, Ulm University, and ^bDepartment of Internal Medicine II – Cardiology, Ulm University Medical Center, Ulm, and ^cBaden-Württemberg State Health Office, Stuttgart, Germany

Key Words

Atopy · Apolipoprotein AI · Apolipoprotein B · Asthma · Schoolchildren

Abstract

Background: There is evidence for an association between serum cholesterol concentrations and asthma, but little is known about the underlying mechanisms. We explored the associations between serum apolipoprotein concentrations and symptoms of asthma and atopy. **Methods:** In a population-based cross-sectional study among 10-year-old schoolchildren (n = 462), plasma concentrations of apolipoprotein AI (apoAI) and apolipoprotein B (apoB) were measured. Information on disease symptoms and diagnoses was collected by parental questionnaire. Multivariate logistic regression models were used to analyse the data. **Results:** High plasma apoAI concentrations (≥ 1.74 g/l) were associated with high prevalence of wheeze (OR 3.65; 95% CI 1.43–9.33), and a trend was seen with asthma (OR 3.35; 95% CI 0.94–11.93). In linear analyses, plasma apoAI concentrations were positively related to wheeze (β -coefficient = 3.20; $p = 0.045$) and non-atopic wheeze (β -coefficient = 4.47; $p = 0.036$), and a trend was found for asthma (β -coefficient = 3.29; $p = 0.099$). Overall, plasma apoB concentrations were not associated with either symptoms of asthma or allergy. **Conclusion:** Our results support the hypothesis that high apoAI is associated

with the manifestation of asthma and atopy. The underlying pathomechanism still remains to be fully elucidated, but suggests that immune mechanisms may play a central role. However, our results are based on a small study sample and larger studies are warranted to confirm these observations.

Copyright © 2009 S. Karger AG, Basel

Introduction

Western lifestyle and, in particular, dietary habits such as fatty acid intake are thought to contribute to the worldwide increase in asthma [1, 2]. Current evidence indicates that lipids, in particular polyunsaturated fatty acids (PUFA), play an important role in asthma manifestation as well as in other respiratory and allergic symptoms [1, 3]. Potential mechanisms have been proposed by which dietary fatty acids affect asthma and allergies, such as changes in the composition of the cell membrane, production of the immune mediators prostaglandins and leukotrienes, and their effects on the regulation of Th1 and Th2 cytokines [4]. The Th1-Th2 paradigm has been established to describe immune regulation based on T-

¹ Stephan K. Weiland, head of the Institute of Epidemiology, Ulm University, died on March 19, 2007.

cell differentiation and cytokine profiles. Asthma and allergy are characterized by a Th2-dominant response, which is characterized by the production of interleukin (IL)-4, IL-5, IL-13 and IL-10 [5]. In experimental studies, for instance, high plasma cholesterol concentrations enhance the expression of pro-inflammatory genes and cytokines [6, 7]. Results from epidemiological studies suggest that serum cholesterol concentrations are associated with asthma and allergies in children [8, 9] and adults [9–11].

During recent years, studies on cardiovascular outcomes have suggested that apolipoproteins may be more sensitive indicators for cardiovascular disease than plasma lipid [high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C)] concentrations [12, 13]. The lipid transport system can be divided into 2 main subtypes: the apolipoprotein B (apoB) system, including very low-density lipoprotein (VLDL), intermediate-density lipoprotein, large buoyant (LDL-C) and small dense LDL-C, and the apolipoprotein AI (apoAI) system, including HDL-C. ApoAI is secreted by the liver and present in the majority of HDL-C particles [14]. ApoAI is highly correlated with anti-atherogenic HDL-C particles [15].

ApoB 100 indicates the number of potentially atherogenic lipoprotein particles such as VLDL, intermediate-density lipoprotein, LDL-C and small dense LDL-C [16]. Larger apoB-containing particles can enhance the fibrinolytic system by inhibition of the endogenous fibrinolysis and by stimulating cytokine production and inflammatory reactions.

Only a few studies have investigated cholesterol concentrations in relation to asthma and allergies, but to the best of our knowledge, no association between apolipoproteins and asthma, rhinoconjunctivitis and eczema in children has been reported yet [9–11]. Apolipoproteins as markers of the lipoprotein composition (LDL-C and HDL-C) may provide further insight into the relationship between lipid metabolism and disease. We sought to explore the associations of plasma apoB and apoAI concentrations with respiratory and atopic disease.

Subjects and Methods

Study Population

As part of a surveillance programme of chronic disorders (including asthma and symptoms of atopy) in children, a cross-sectional study was carried out with the assistance of local health departments. The study was coordinated by the Baden-Württemberg State Health Office and approved by the local ethics commit-

tees [17]. Six schools in the State of Baden-Württemberg were randomly selected and 934 schoolchildren (4th grade) were invited to participate in the study. Between October 2004 and March 2005, 557 pupils (60%) were recruited in South-West Germany after written informed consent had been obtained from the parents. We used data of 462 children (50%) with available blood samples to investigate the relationship between plasma apolipoprotein concentrations and asthma, rhinoconjunctivitis and eczema.

Outcome Variables

Parental questioning by means of a standardized questionnaire based on the International Study on Allergies and Asthma (ISAAC) was performed [18]. Information on symptoms of asthma, rhinoconjunctivitis and eczema as well as on lifestyle variables were collected.

Past year prevalence of asthma or atopy was assessed by the following questions: 'Has your child had wheezing or whistling in the chest in the past 12 months?' For rhinoconjunctivitis, the following questions were used: 'Has your child had a problem with sneezing or a running or blocked nose?' and 'In the past 12 months, has this nose problem been accompanied by itchy watery eyes?' Atopic dermatitis referred to the questions: 'Has your child ever had an itchy rash, which was coming and going for at least six months? Has the itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?' Moreover, self-reported data on physician-diagnosed asthma, hay fever and eczema were collected. In combination with wheeze during the past year, symptoms of rhinoconjunctivitis were used as a proxy for atopy [19]. Wheeze in the presence of rhinoconjunctivitis was defined as atopic wheeze, and without rhinoconjunctivitis, as non-atopic wheeze.

Covariates

All children were invited to a physical examination, where the height and weight were measured in a standardized manner. The body mass index (BMI) was calculated as weight (kg)/height² (m). The question: 'Does a smoker live in your home?' was used for environmental tobacco smoke exposure (yes, no). Information on familial atopy was collected by asking whether one of the family members had/has atopic disease. Data on birth weight was addressed using the question: 'How much did your child weigh at birth?' and classified as <2,000 g, ≥2,000 g, or missing values.

Laboratory Methods

In 462 children, non-fasting EDTA blood was drawn. After centrifugation, samples were aliquoted and stored at –80°C until analysis. All laboratory analyses were performed blinded to these data in the Biomarker Laboratory at the Department of Internal Medicine II – Cardiology, Ulm University Medical Center.

ApoAI and apoB were measured by immunonephelometry on a BN II analyser (Dade Behring, Marburg, Germany). The inter-assay coefficient of variation for apoAI at 1.73 g/l was 6.7% (n = 6) and the corresponding coefficient of variation for apoB at 0.92 g/l was 4.6% (n = 6).

For the analysed biomarkers, no accepted cut-off points in children exist. Therefore, we chose the 90th percentiles of apoAI and apoB in our data as cut-off points (≥1.74 and ≥0.88 g/l, respectively) and compared subjects with those who had lower plasma concentrations.

Table 1. Demographic and laboratory characteristics of 462 schoolchildren by asthma, rhinoconjunctivitis and eczema

Variables	Total (N = 462)	Wheeze past year (N = 447, n = 30)	Asthma physi- cian diagnosed (N = 375, n = 22)	Rhinoconjunc- tivitis past year (N = 458, n = 41)	Atopic wheeze past year (N = 398, n = 11)	Hay fever physi- cian diagnosed (N = 379, n = 32)	Atopic dermatitis (N = 388, n = 50)	Eczema physi- cian diagnosed (N = 390, n = 72)
<i>Continuous</i>								
Age, years								
Median	10.00	9.90	9.95	9.80	9.70	9.85	10.00	9.90
Q1, Q3	9.60, 10.30	9.70, 10.30	9.60, 10.30	9.60, 10.10	9.40, 10.10	9.70, 10.25	9.60, 10.40	9.70, 10.30
BMI								
Median	17.12	17.04	18.02	17.53	15.48	17.44	17.71	16.86
Q1, Q3	15.52, 19.29	14.79, 19.23	16.14, 20.78	15.09, 19.77	14.74, 18.72	15.84, 19.41	16.00, 20.19	15.02, 19.11
ApoAI, g/l								
Median	1.49	1.53	1.50	1.52	1.52	1.53	1.48	1.54
Q1, Q3	1.39, 1.62	1.39, 1.78	1.38, 1.64	1.38, 1.64	1.38, 1.86	1.39, 1.64	1.31, 1.64	1.39, 1.64
ApoB, g/l								
Median	0.68	0.67	0.65	0.66	0.66	0.72	0.67	0.70
Q1, Q3	0.59, 0.79	0.57, 0.85	0.57, 0.78	0.58, 0.77	0.59, 0.84	0.61, 0.84	0.61, 0.78	0.60, 0.80
<i>Categorical</i>								
Girls, %	53.1	36.7*	36.4	41.5	36.4	56.3	64.0	55.6
Environmental tobacco smoke, %	47.5	50.0	35.0	41.5	36.4	37.5	46.9	37.5
Familiar atopy, %	36.9	60.0**	68.2***	59.0*	63.6*	53.3**	68.8***	53.5***

N = Total number of subjects studied; n = number of patients with the corresponding symptoms.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Statistical Analysis

Means and percentiles were calculated using the SAS procedure UNIVARIATE. A Mann-Whitney U test was used to assess differences of continuous variables between groups (SAS procedure NPARIWAY) and the χ^2 test for proportions, respectively. Logistic regression analysis was used to calculate multivariate odds ratios (OR) and their 95% confidence intervals (CI) for symptoms of asthma and atopy by plasma concentrations of apolipoproteins. Models were adjusted for sex, environmental tobacco smoke (yes, no), BMI, birth weight ($<2,000$ g, $\geq 2,000$ g, or missing) and familiar atopy (yes, no). In addition, sex-stratified analyses were performed. Age did not influence the estimates substantially and was therefore not included in the final models. Models with continuous values of the log-transformed exposure variables (apoAI, apoB) were calculated in order to examine the consistency of the data. Interactions were tested using the Wald test. All provided p values are 2-sided. The statistical software package SAS release 9.1 (SAS Institute, Cary, N.C., USA) was used.

Results

Demographic and laboratory characteristics of the study population are shown in table 1. The mean age was 10.0 (SD 0.6) years. Overall, 462 children (47% boys, 53% girls) were included in the study, of which 30 (6.7%) had

experienced at least 1 episode of wheeze during the past year and 30 (6.7%) were ever diagnosed with asthma. In addition, 50 (12.9%) reported symptoms of atopic dermatitis and 81 (18.2%) children had ever been diagnosed with eczema. Fewer girls than boys suffered from wheeze during the past year. Familiar atopy was statistically significantly associated with all investigated disease outcomes. Exposure to environmental tobacco smoke was not associated with any disease outcome.

Table 2 shows the associations of plasma apoAI concentrations with asthma and symptoms of atopy. In multivariate models, high apoAI concentrations were statistically significantly associated with wheeze during the past year (OR 3.65; 95% CI 1.43–9.33), whereas for the prevalence of physician-diagnosed asthma, only a trend, but no statistically significant association, was found (OR 3.35; 95% CI 0.94–11.93). Further adjustment for BMI slightly strengthened the association for non-atopic wheeze. In a model using apoAI as a log-transformed continuous variable, increasing plasma apoAI concentrations were associated with increasing prevalence of wheeze (β -coefficient = 3.20; $p = 0.045$) and, particularly, with non-atopic wheeze (β -coefficient = 4.47; $p = 0.036$). For physician-

Table 2. Associations of plasma apoAI concentrations (≥ 90 th percentile vs. < 90 th percentile) with asthma, rhinoconjunctivitis and eczema

	n/N	Multivariate ¹	Multivariate ¹		Multivariate ²		Continuous values ^{2,3} log apoAI	
			< 90 th percentile, OR		≥ 90 th percentile		β -coefficient	p value
			OR	95% CI	OR	95% CI		
Wheeze, past year	30/422	1	3.65	1.46–9.13	3.65	1.43–9.33	3.20	0.045
Atopic wheeze	11/382	1	4.16	1.00–17.36	3.99	0.93–17.06	2.86	0.281
Non-atopic wheeze	19/390	1	4.04	1.28–12.74	4.33	1.33–14.10	4.47	0.036
Asthma, physician diagnosed	20/361	1	3.06	0.88–10.59	3.35	0.94–11.93	3.29	0.099
Rhinoconjunctivitis	39/438	1	2.27	0.91–5.66	2.48	0.97–6.34	1.33	0.359
Hay fever, physician diagnosed	32/365	1	1.37	0.38–4.95	1.41	0.38–5.17	1.95	0.233
Symptoms of atopic dermatitis	47/373	1	1.08	0.34–3.43	1.11	0.35–3.58	0.62	0.648
Eczema, physician diagnosed	71/375	1	1.48	0.62–3.53	1.31	0.55–3.18	0.93	0.421

n = Number of patients with the corresponding symptoms; N = total number of subjects studied.

¹ Adjusted for sex, familiar atopy, birth weight and environmental tobacco smoking.

² Adjusted for sex, familiar atopy, birth weight, environmental tobacco smoking and BMI in tertiles.

³ log-transformed.

diagnosed asthma (β -coefficient = 3.29; $p = 0.099$), a trend was found. No further associations between plasma apoAI and disease outcomes were observed.

In table 3, the associations of plasma apoB concentrations with asthma and symptoms of atopy are shown. In multivariate models, high apoB concentrations were neither associated statistically significantly with wheeze during the past year (OR 2.30; 95% CI 0.85–6.24) nor with non-atopic wheeze (OR 2.68; 95% CI 0.80–8.97). Consistent with the results for categorical analysis of apoB plasma concentrations, linear analyses revealed no association with wheeze during the past year or in subgroups. No associations were found between plasma apoB concentrations and symptoms of atopy. In general linear models (data not shown), the adjusted means of apoAI differed statistically significantly by wheeze ($p = 0.003$), non-atopic wheeze ($p = 0.002$) and physician-diagnosed asthma ($p = 0.021$). Adjusted means of plasma apoB concentrations differed only by non-atopic wheeze ($p = 0.044$). In sex-specific analyses (table 4), plasma apoAI concentrations were more strongly associated with rhinoconjunctivitis in boys than in girls. Concerning plasma apoB concentrations, stratification by sex strengthened the associations for wheeze during the past year among girls and for eczema among boys. When the categorical variables of apoAI and apoB were analysed, there was no significant effect modification by sex. Finally, the apoB/apoAI ratio was not associated with symptoms of asthma and allergy (data not shown).

Discussion

Our observation that high apoAI concentrations are associated with non-atopic wheeze during the past year is consistent with former research showing a positive association between HDL-C concentrations and asthma and allergies [10, 20]. In a cohort study, similar HDL-C concentrations among allergic and non-allergic children at the age of 5 years were found, except for higher concentrations among children with recurrent wheeze [9]. In an outpatient clinic-based study among adults, hypercholesterolaemia was associated with asthma [11]. Experimental studies have shown that cholesterol enhances pulmonary eosinophilic inflammation [6]. It is believed that cholesterol increases the risk of asthma by promoting a proinflammatory state in the bronchial airway [21]. Research in cardiovascular disease has shown that the anti-inflammatory properties of HDL-C may become proinflammatory, in particular in the presence of a chronic systemic inflammatory response [22, 23]. Concerning asthma and allergies, increasing apoAI concentrations could indicate the release of IL-4, IL-13 and IL-5 by Th2 cells, which is known to activate mast cells, eosinophils and basophils and promote the production of IgE by B cells. In contrast, interferon (IFN)- γ -producing Th1 cells may protect against allergic disease by reducing the activity of Th2 effector cells [24]. Experimental research revealed that apoAI inhibits the tumor necrosis factor- α and IL-1 β production through inhibition of contact-acti-

Table 3. Associations of plasma apoB concentrations (≥ 90 th percentile vs. < 90 th percentile) with asthma, rhinoconjunctivitis and eczema

	n/N	Multivariate ¹			Multivariate ²		Continuous values ^{2,3} log apoB	
		< 90 th per- centile, OR	≥ 90 th percentile		≥ 90 th percentile		β -coefficient	p value
			OR	95% CI	OR	95% CI		
Wheeze, past year	30/422	1	2.17	0.81–5.76	2.30	0.85–6.24	1.12	0.222
Atopic wheeze	11/377	1	1.80	0.37–8.91	1.91	0.38–9.56	0.30	0.835
Non-atopic wheeze	19/390	1	2.58	0.78–8.50	2.68	0.80–8.97	1.51	0.191
Asthma, physician diagnosed	20/361	1	0.83	0.18–3.81	0.79	0.17–3.67	–0.47	0.662
Rhinoconjunctivitis	37/431	1	1.18	0.43–3.24	1.27	0.46–3.55	–0.46	0.558
Hay fever, physician diagnosed	32/365	1	1.08	0.35–3.29	1.05	0.34–3.27	0.76	0.399
Atopic dermatitis	47/367	1	1.01	0.36–2.86	0.96	0.34–2.72	0.05	0.950
Eczema, physician diagnosed	71/375	1	1.57	0.71–3.22	1.76	0.80–3.86	0.27	0.673

n = Number of patients with the corresponding symptoms; N = total number of subjects studied.

¹ Adjusted for sex, familiar atopy, birth weight and environmental tobacco smoking.

² Adjusted for sex, familiar atopy, birth weight, environmental tobacco smoking and BMI in tertiles.

³ log-transformed.

Table 4. Associations of plasma apoAI and apoB concentrations with asthma, rhinoconjunctivitis and eczema by sex

	n/N	apoAI			apoB		
		<90th per- centile, OR	≥90th percentile		<90th per- centile, OR	≥90th percentile	
			OR	95% CI		OR	95% CI
Girls							
Wheeze, past year	11/226	1	3.63	0.81–16.21	1	4.85	1.08–21.71
Asthma, physician diagnosed	7/187	1	NA		1	NA	
Rhinoconjunctivitis	15/229	1	0.65	0.08–5.51	1	1.48	0.30–7.35
Atopic dermatitis	30/202	1	1.18	0.22–6.41	1	0.87	0.22–3.46
Eczema, physician diagnosed	39/196	1	0.70	0.14–3.45	1	1.03	0.34–3.19
Boys							
Wheeze, past year	19/196	1	3.66	1.08–12.49	1	1.37	0.35–5.44
Asthma, physician diagnosed	13/168	1	5.22	1.15–23.70	1	1.46	0.28–7.75
Rhinoconjunctivitis	22/202	1	4.52	1.42–14.46	1	1.12	0.29–4.33
Atopic dermatitis	17/155	1	1.07	0.21–5.53	1	0.98	0.19–5.00
Eczema, physician diagnosed	32/173	1	1.71	0.54–5.40	1	3.63	1.10–11.97

Groups adjusted for familiar atopy, birth weight, BMI in tertiles and environmental tobacco smoking. n = Number of patients with the corresponding symptoms; N = total number of subjects studied; NA = not assessed.

vated monocytes [25], indicating an anti-inflammatory effect in the context of infection. It can be speculated whether inhibition of the Toll-like receptor activation and Th1 response contribute to the positive association between apoAI and wheeze [26].

In our study, the strength of the association between apoAI, as a substitute for HDL-C, and wheeze during the past year was independent of the atopic status. Concerning

objective markers of atopy, Schäfer et al. [10] found statistically significant inverse associations for the skin prick test reactivity with concentrations of total cholesterol and LDL-C, and a borderline statistically significant association with HDL-C, while for radioallergosorbent test reactivity, only the association with total cholesterol reached statistical significance. However, in their study, both skin prick test and radioallergosorbent test reactivity were as-

sociated with PUFA concentrations [10], suggesting that fatty acid composition may be relevant for the disease onset [20, 27]. Previous research has focused on the relationship between dietary fatty acid intake, asthma and symptoms of atopy only [1, 3, 8, 10, 11, 20, 28], which limits comparability. Particularly, PUFA have been investigated as precursors of the eicosanoids, which are involved in immune modulation [29]. In vitro research has shown that reduced HDL-C concentrations may be accompanied by a reduced ability to respond to lipopolysaccharides [30]. Clinically, infection and inflammation are associated with a reduction in HDL-C and apoAI concentrations [31]. It can be speculated whether changes in the antioxidant properties [12, 32] or loss of the antioxidant capacity of apoAI [12, 33] contribute to the observed associations. In children with severe respiratory syncytial virus infection, the production of INF- γ has been reduced, suggesting Th1 involvement towards Th2 dominance [34]. Supporting results were reported from an in vitro study, in which T cells stimulated by apoAI-pretreated dendritic cells produced significantly lower levels of INF- γ , suggesting a shift towards Th2 response and allergy [35].

Consistent with an earlier report, we observed differential associations with symptoms of lung and skin manifestations [36]. In our population, atopic dermatitis and rhinoconjunctivitis were not associated with apoAI concentrations, whereas other authors found increased HDL-C concentrations to be associated with positive skin prick tests or allergic sensitization [10, 37]. However, it has also been reported that HDL-C was associated with decreased risk of allergic sensitization in a cross-sectional study among adolescents [38], suggesting that low apoAI concentrations may represent a potential risk factor for allergic skin disease.

Since apoB represents a marker for more dense and potentially atherogenic lipoprotein particles and gives an estimate on LDL-C particle numbers, our observations support the hypothesis that disorders in the lipid metabolism, such as hypercholesterolaemia [10], or fatty acid composition [20, 28], may be associated with asthma [1, 8]. Our observation of a lack of an association between apoB and wheeze is consistent with earlier results on asthma [10, 11]. However, our results suggest that high apoB concentrations might be associated with non-atopic wheeze, indicating a relationship between apoB concentrations and inflammation. Though several plausible pathomechanisms such as the inhibition of cytokine production and antioxidant function have been proposed [12, 21], none is solely conclusive. Our observations of no overall association between apoB and eczema are in con-

trast with the results of a case-control study among German adults, in which an inverse relationship between increasing LDL-C concentration and atopic eczema was found [10]. However, their results were not adjusted for potential confounders.

A possible explanation for the lack of an association between the apoB/apoAI ratio and asthma and atopy in our data could be the small numbers and differential mechanisms according to asthma phenotype. Inconclusive results on the serum lipid ratio using measurement of n-6 and n-3 fatty acids in serum phospholipids in adults have also been reported by other authors [39].

Concerning sex differences, our results of a stronger association of apoAI concentrations with rhinoconjunctivitis in boys are in contrast to earlier results reporting a significant positive association between HDL-C concentrations and allergic rhinitis in women only [10]. Pesonen et al. [9] reported no statistically significant association between increased HDL-C concentrations and subsequent allergies in children at the age of 5 years, but no gender-stratified analyses have been performed. We found stronger associations between high apoB concentrations and wheeze during the past year among girls than among boys, suggesting that sex differences in the regulation of apoB may be relevant. Consistently, sex differences in the apoB distribution were found among Spanish adolescents [40]. These findings are in line with the observation that oestrogens are inversely correlated with LDL-C and VLDL concentrations [41]. Indeed, results from epidemiological studies suggest sex-specific associations between dietary fat intake and asthma in children [8] and adults [10, 28]. Results from a prospective birth cohort study indicate that sex-specific patterns of allergic disease could be related to sex-specific cytokine expression [42]. A wheezing phenotype at the age of 3 years was characterized by increased IFN- γ , IL-5 and IL-13 response in boys and decreased IFN- γ concentrations in girls [43]. In vitro application of oestrogen and progesterone to human peripheral mononuclear cells resulted in the production of Th2 cytokines IL-4 and IL-13 [44]. However, it is still poorly understood which mechanisms contribute to the sex-specific pattern among children and adults. One hypothesis suggests that sex hormones are not only linked to lipid metabolism but also to immune functions [45]. Due to the small number of children with disease symptoms in the strata, it has to be kept in mind that these results may have occurred by chance.

The strengths of our study include the measurement of biological markers for exposure, the adjustment for potential confounders and its population-based approach.

In addition, the well-established ISAAC questionnaire has been used [18]. Several limitations of the study need to be considered. In bivariate analysis, we found no association between environmental tobacco smoke exposure and any of the disease outcomes. A possible explanation might be that among parents with asthma or allergy, there were fewer smokers, whereas asthma and allergy were more prevalent among the offsprings. In addition, the number of smoked cigarettes rather than the smoking status per se could be associated with asthma and atopy. However, the sample size of this explorative study was relatively small, which limits statistical power. Under the assumption of a case-control study with a power of 0.80 and a standard error of 0.05, a group size of 109 children each would have been required in order to detect differences of prevalence (0.05 and 0.17) as observed for elevated apoAI concentrations and wheeze.

No objective measurements for allergic sensitization and bronchial hyper-responsiveness were available in our study. However, we applied a well-established instrument on disease outcome among children [18]. Also, acute infections in children were found to be accompanied by enhanced oxidative modification of LDL-C and by a decrease in plasma HDL-C [46]. The exclusion of 8 children with C-reactive protein values >10 mg/l did not substantially alter our estimates, indicating that acute inflammation may have only slightly contributed to the observed estimates. In addition, the cross-sectional study design precludes conclusions regarding causality, since data on exposure and outcome were collected at the same time.

Residual confounding due to dietary fat composition cannot be excluded either. Other dietary factors such as

fat-soluble vitamin E may further contribute to the observed associations [47]. However, further adjustment for paternal school education did not substantially alter the estimates, as did adjustment for breastfeeding or number of older siblings. ApoAI and apoB have been shown to be strong predictors of cardiovascular disease outcomes [13, 48], suggesting that they better capture the adverse effects of serum lipids than plasma HDL-C and LDL-C concentrations, which are determined by number and volume of particle size. However, other bioactive lipids, which are mainly transported by apoAI, may have contributed to the observed association with wheeze.

In conclusion, our results support the hypothesis that disturbances in the lipoprotein profiles, in particular elevated apoAI, are related to asthma and allergy. ApoAI and in trend apoB were differently associated with disease outcome, suggesting different pathomechanisms. Whether apolipoproteins per se due to their anti-inflammatory function or rather due to the composition of fatty acids contribute to disease presentation remains to be elucidated.

Acknowledgements

The authors would like to thank Gerlinde Trischler for her excellent technical assistance, Holger Knebel for documentation and data management, and Anne Katrin Kersten for assisting in data analysis. We would like to thank the staff of the local health departments for organizing the field research and taking the blood samples. Finally, we thank all study participants and their parents.

References

- 1 Fogarty A, Britton J: The role of diet in the aetiology of asthma. *Clin Exp Allergy* 2000; 30:615–627.
- 2 von Mutius E: Influences in allergy: epidemiology and the environment. *J Allergy Clin Immunol* 2004;113:373–379.
- 3 Dunder T, Kuikka L, Turtinen J, Rasanen L, Uhari M: Diet, serum fatty acids, and atopic diseases in childhood. *Allergy* 2001;56:425–428.
- 4 Kankaanpää P, Sutas Y, Salminen S, Lichtenstein A, Isolauri E: Dietary fatty acids and allergy. *Ann Med* 1999;31:282–287.
- 5 Holgate ST: Pathogenesis of asthma. *Clin Exp Allergy* 2008;38:872–897.
- 6 Yeh YF, Huang SL: Dietary cholesterol enhances pulmonary eosinophilic inflammation in a murine model of asthma. *Int Arch Allergy Immunol* 2001;125:329–334.
- 7 Stokes KY, Cooper D, Taylor A, Granger DN: Hypercholesterolemia promotes inflammation and microvascular dysfunction: role of nitric oxide and superoxide. *Free Radic Biol Med* 2002;33:1026–1036.
- 8 Bolte G, Frye C, Hoelscher B, Meyer I, Wjst M, Heinrich J: Margarine consumption and allergy in children. *Am J Respir Crit Care Med* 2001;163:277–279.
- 9 Pesonen M, Ranki A, Siimes MA, Kallio MJ: Serum cholesterol level in infancy is inversely associated with subsequent allergy in children and adolescents. A 20-year follow-up study. *Clin Exp Allergy* 2008;38:178–184.
- 10 Schafer T, Ruhdorfer S, Weigl L, Wessner D, Heinrich J, Doring A, Wichmann HE, Ring J: Intake of unsaturated fatty acids and HDL cholesterol levels are associated with manifestations of atopy in adults. *Clin Exp Allergy* 2003;33:1360–1367.
- 11 Al Shawwa B, Al Huniti N, Titus G, Abu-Hasan M: Hypercholesterolemia is a potential risk factor for asthma. *J Asthma* 2006;43: 231–233.
- 12 Walldius G, Jungner I: Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. *J Intern Med* 2004;255:188–205.

- 13 Thompson A, Danesh J: Associations between apolipoprotein B, apolipoprotein AI, the apolipoprotein B/AI ratio and coronary heart disease: a literature-based meta-analysis of prospective studies. *J Intern Med* 2006; 259:481–492.
- 14 Lewis GF, Rader DJ: New insights into the regulation of HDL metabolism and reverse cholesterol transport. *Circ Res* 2005;96: 1221–1232.
- 15 Walldius G, Jungner I: The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy – a review of the evidence. *J Intern Med* 2006;259:493–519.
- 16 Elovson J, Chatterton JE, Bell GT, Schumaker VN, Reuben MA, Puppione DL, Reeve JR Jr, Young NL: Plasma very low density lipoproteins contain a single molecule of apolipoprotein B. *J Lipid Res* 1988;29:1461–1473.
- 17 Zollner IK, Weiland SK, Piechotowski I, Gabrio T, von Mutius E, Link B, Pfaff G, Kourou B, Wuthe J: No increase in the prevalence of asthma, allergies, and atopic sensitisation among children in Germany: 1992–2001. *Thorax* 2005;60:545–548.
- 18 Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW: International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483–491.
- 19 Braun-Fahrlander C, Wuthrich B, Gassner M, Grize L, Sennhauser FH, Varonier HS, Vuille JC: Validation of a rhinitis symptom questionnaire (ISAAC core questions) in a population of Swiss school children visiting the school health services. SCARPOL-team. Swiss Study on Childhood Allergy and Respiratory Symptom with respect to Air Pollution and Climate. *International Study of Asthma and Allergies in Childhood. Pediatr Allergy Immunol* 1997;8:75–82.
- 20 Bolte G, Kompauer I, Fobker M, Cullen P, Keil U, Mutius E, Weiland SK: Fatty acids in serum cholesteryl esters in relation to asthma and lung function in children. *Clin Exp Allergy* 2006;36:293–302.
- 21 Burger D, Dayer JM: High-density lipoprotein-associated apolipoprotein A-I: the missing link between infection and chronic inflammation? *Autoimmun Rev* 2002;1:111–117.
- 22 Van Lenten BJ, Hama SY, de Beer FC, Stafforini DM, McIntyre TM, Prescott SM, La Du BN, Fogelman AM, Navab M: Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. *J Clin Invest* 1995;96:2758–2767.
- 23 Ansell BJ, Watson KE, Fogelman AM, Navab M, Fonarow GC: High-density lipoprotein function: recent advances. *J Am Coll Cardiol* 2005;46:1792–1798.
- 24 Holgate ST: Pathogenesis of asthma. *Clin Exp Allergy* 2008;38:872–897.
- 25 Hyka N, Dayer JM, Modoux C, Kohno T, Edwards CK III, Roux-Lombard P, Burger D: Apolipoprotein A-I inhibits the production of interleukin-1beta and tumor necrosis factor-alpha by blocking contact-mediated activation of monocytes by T lymphocytes. *Blood* 2001;97:2381–2389.
- 26 Shamshiev AT, Ampenberger F, Ernst B, Rohrer L, Marsland BJ, Kopf M: Dyslipidemia inhibits Toll-like receptor-induced activation of CD8alpha-negative dendritic cells and protective Th1 type immunity. *J Exp Med* 2007;204:441–452.
- 27 Hoff S, Seiler H, Heinrich J, Kompauer I, Nieters A, Becker N, Nagel G, Gedrich K, Karg G, Wolfram G, Linseisen J: Allergic sensitisation and allergic rhinitis are associated with n-3 polyunsaturated fatty acids in the diet and in red blood cell membranes. *Eur J Clin Nutr* 2005;59:1071–1080.
- 28 Nagel G, Linseisen J: Dietary intake of fatty acids, antioxidants and selected food groups and asthma in adults. *Eur J Clin Nutr* 2005; 59:8–15.
- 29 Calder PC: n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* 2006;83:1505S–1519S.
- 30 Thompson PA, Kitchens RL: Native high-density lipoprotein augments monocyte responses to lipopolysaccharide (LPS) by suppressing the inhibitory activity of LPS-binding protein. *J Immunol* 2006;177:4880–4887.
- 31 Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C: Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. *J Lipid Res* 2004;45:1169–1196.
- 32 Pruzanski W, Stefanski E, de Beer FC, de Beer MC, Ravandi A, Kuksis A: Comparative analysis of lipid composition of normal and acute-phase high density lipoproteins. *J Lipid Res* 2000;41:1035–1047.
- 33 Van Lenten BJ, Reddy ST, Navab M, Fogelman AM: Understanding changes in high density lipoproteins during the acute phase response. *Arterioscler Thromb Vasc Biol* 2006;26:1687–1688.
- 34 Renzi PM, Turgeon JP, Marcotte JE, Drblik SP, Berube D, Gagnon MF, Spier S: Reduced interferon-gamma production in infants with bronchiolitis and asthma. *Am J Respir Crit Care Med* 1999;159:1417–1422.
- 35 Kim KD, Lim HY, Lee HG, Yoon DY, Choe YK, Choi I, Paik SG, Kim YS, Yang Y, Lim JS: Apolipoprotein A-I induces IL-10 and PGE2 production in human monocytes and inhibits dendritic cell differentiation and maturation. *Biochem Biophys Res Commun* 2005; 338:1126–1136.
- 36 Linneberg A, Simonsen JB, Petersen J, Stensballe LG, Benn CS: Differential effects of risk factors on infant wheeze and atopic dermatitis emphasize a different etiology. *J Allergy Clin Immunol* 2006;117:184–189.
- 37 Shenoi A, Kumar L, Sarihyian S, Gangully NK: High density lipoprotein cholesterol and total cholesterol in children with asthma and allergic rhinitis. *Acta Paediatr* 1992;81: 150–152.
- 38 McKeever TM, Lewis SA, Smit H, Burney P, Britton J, Cassano PA: Serum nutrient markers and skin prick testing using data from the Third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2004;114:1398–1402.
- 39 Kompauer I, Demmelmaier H, Koletzko B, Bolte G, Linseisen J, Heinrich J: n6/n3 hypothesis and allergies: biologically plausible, but not confirmed. *Eur J Med Res* 2004;9: 378–382.
- 40 Ruiz JR, Ortega FB, Moreno LA, Warnberg J, Gonzalez-Gross M, Cano MD, Gutierrez A, Castillo MJ: Reference values for serum lipids and lipoproteins in Spanish adolescents: the AVENA study. *Soz Praventivmed* 2006; 51:99–109.
- 41 Campos H, Walsh BW, Judge H, Sacks FM: Effect of estrogen on very low density lipoprotein and low density lipoprotein subclass metabolism in postmenopausal women. *J Clin Endocrinol Metab* 1997;82:3955–3963.
- 42 Uekert SJ, Akan G, Evans MD, Li Z, Roberg K, Tisler C, Dasilva D, Anderson E, Gangnon R, Allen DB, Gern JE, Lemanske RF Jr: Sex-related differences in immune development and the expression of atopy in early childhood. *J Allergy Clin Immunol* 2006;118: 1375–1381.
- 43 Uekert SJ, Akan G, Evans MD, Li Z, Roberg K, Tisler C, Dasilva D, Anderson E, Gangnon R, Allen DB, Gern JE, Lemanske RF Jr: Sex-related differences in immune development and the expression of atopy in early childhood. *J Allergy Clin Immunol* 2006;118: 1375–1381.
- 44 Hamano N, Terada N, Maesako K, Hohki G, Ito T, Yamashita T, Konno A: Effect of female hormones on the production of IL-4 and IL-13 from peripheral blood mononuclear cells. *Acta Otolaryngol Suppl* 1998;537:27–31.
- 45 Fish EN: The X-files in immunity: sex-based differences predispose immune responses. *Nat Rev Immunol* 2008;8:737–744.
- 46 Liuba P, Persson J, Luoma J, Yla-Herttuala S, Pesonen E: Acute infections in children are accompanied by oxidative modification of LDL and decrease of HDL cholesterol, and are followed by thickening of carotid intima-media. *Eur Heart J* 2003;24:515–521.
- 47 McKeever TM, Britton J: Diet and asthma. *Am J Respir Crit Care Med* 2004;170:725–729.
- 48 Meisinger C, Loewel H, Mraz W, Koenig W: Prognostic value of apolipoprotein B and A-I in the prediction of myocardial infarction in middle-aged men and women: results from the MONICA/KORA Augsburg cohort study. *Eur Heart J* 2005;26:271–278.