

ORIGINAL ARTICLE

Few associations between high-sensitivity C-reactive protein and environmental factors in 4.5-year-old children

Kirsi Mustonen¹, Leea Keski-Nisula^{1,2}, Outi Vaarala³, Petra Ina Pfefferle⁴, Harald Renz⁴, Josef Riedler⁵, Jean-Charles Dalphin^{6,7}, Gisela Buechele⁸, Roger Lauener^{9,10}, Charlotte Braun-Fahrländer^{11,12}, Erika von Mutius¹³, Juha Pekkanen^{1,14} & the PASTURE Study Group*

¹Environmental Health Department, National Institute for Health and Welfare, Kuopio, Finland; ²Department of Obstetrics and Gynecology, Kuopio University Hospital, Kuopio, Finland; ³Immune Response Unit, Department of Vaccination and Immune Protection, National Institute for Health and Welfare, Helsinki, Finland; ⁴Institute for Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, Philipps-University of Marburg, Marburg, Germany; ⁵Children and Young Adults' Medicine, Children's Hospital, Schwarzach, Austria; ⁶UMR6249, Laboratoire Chrono-Environnement, Université de Franche-Comté, Besançon, France; ⁷Department of Respiratory Disease, University of Hospital of Besançon, Besançon, France; ⁸Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany; ⁹Children's Hospital, and Christine Kühne-Center for Allergy Research and Education, University of Zürich, Zürich, Switzerland; ¹⁰Children's Allergy and Asthma Hospital, Hochgebirgsklinik, and Christine Kühne-Center for Allergy Research and Education, Davos, Switzerland; ¹¹Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland; ¹²Faculty of Medicine, University of Basel, Basel, Switzerland; ¹³Dr. von Hauner Children's Hospital of the Ludwig Maximilian University Munich, Munich, Germany; ¹⁴Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland

To cite this article: Mustonen K, Keski-Nisula L, Vaarala O, Pfefferle PI, Renz H, Riedler J, Dalphin J-C, Buechele G, Lauener R, Braun-Fahrländer C, von Mutius E, Pekkanen J and the PASTURE Study Group. Few associations between high-sensitivity C-reactive protein and environmental factors in 4.5-year-old children. *Pediatr Allergy Immunol* 2012; **00**.

Keywords

hsCRP; specific IgE; allergic disease; atopic sensitization; farming environment; children.

Correspondence

Kirsi M. Mustonen, Department of Environmental Health, National Institute for Health and Welfare, PO Box 95, FIN-70701 Kuopio, Finland.

Tel.: +358 20 610 6337

Fax: +358 20 610 6498

E-mail: kiirsi.mustonen@thl.fi

*The PASTURE Study Group: Anne Karvonen, Marjut Roponen, Anne Hyvärinen, Maija-Riitta Hirvonen, Pekka Tiittanen and Sami Remes (Finland); Virginie Kaulek and Marie-Laure Dalphin (France); Markus Ege, Martin Depner, Juliane Weber, Bianca Schaub, Jon Genuneit and Michael Kabesch (Germany); Sondhja Bitter, George Loss, Caroline Roduit and Remo Frei (Switzerland); Gert Doekes (The Netherlands).

Accepted for publication 6 March 2012

DOI:10.1111/j.1399-3038.2012.01306.x

Abstract

Background: Low-grade inflammation in early childhood might protect from allergic diseases later in life. Our aim was to examine the effects of different environmental factors on low-grade inflammation measured with serum high-sensitivity C-reactive protein (hsCRP) at the age of 4.5 years.

Methods: The high-sensitivity CRP values ($n = 653$) and serum-specific IgE concentrations were measured from 4.5-year-old children in rural areas in five European countries (Austria, Finland, France, Germany, and Switzerland). Children belonged to the prospective multi-center PASTURE birth cohort. Data on early and current farming environment and domestic animal exposure were collected by questionnaires.

Results: Females as well as obese or very obese children at age of 4.5 years had higher hsCRP values than males (aOR 1.84 95% CI 1.27–2.66) and healthy weight children (aOR 4.47 95% CI 1.94–10.31), respectively. Levels were lowest in the summer. Few associations were detected between hsCRP values and farm environmental factors or atopic sensitization. However, there was evidence of children with low levels of hsCRP (below the detection limit) who had increased prevalence of sensitization to inhaled and seasonal allergens. Among non-sensitized children, spending time in stables was associated with reduced hsCRP (15 min–10 h aOR 0.40 95% CI 0.16–0.96 and ≥ 10 h aOR 0.25 95% CI 0.07–0.90), and among sensitized children, maternal smoking was associated with higher hsCRP values (aOR 2.51 95% CI 1.12–5.59).

Conclusion: We found few associations between early environmental farming factors and hsCRP levels, and between hsCRP levels and atopic sensitization in 4.5-year-old children. However, our results suggest that the role played by the environmental factors in low-grade inflammation may differ between sensitized and non-sensitized children.

High-sensitivity C-reactive protein (hsCRP) is a sensitivity marker of low-grade inflammation. Increased levels of hsCRP have been associated with obesity and cardiometabolic risk factors, but most of these studies have been performed in adults with fewer studies available involving children (1–4). During childhood, increased hsCRP levels have also been associated with female sex (5), respiratory impairment (6), asthma severity (7), and with an atherogenic lipid profile in type 1 diabetes (8).

On the other hand, Marschan et al. (9) found that increased concentration of plasma CRP in early infancy (age of 6 months) was associated with a decreased risk of eczema at the age of 2 years. In the study, infants who were treated with probiotic bacteria had higher plasma CRP levels, but the decreased risk of eczema was also seen in children with higher hsCRP levels on placebo (9). Further, it was recently suggested that *Lactobacillus GG* (LGG) treatment in infants with atopic eczema-dermatitis syndrome (AEDS) might induce systemically detectable low-grade inflammation leading to alleviated symptoms of AEDS (10). Both of these studies suggest that a low-grade inflammation in infancy would generate some protection against allergic diseases, at least against eczema (9).

There are several studies that have shown the protective effect of a farming environment in the development of allergic diseases in children (11–14). This protective effect is mostly explained by the abundance and diversity of environmental microbial exposure. However, none study has evaluated whether farming environment would associate with low-grade inflammation itself inducing protective effect against the development of allergic diseases. Thus the aim of this study was to investigate whether environmental factors, such as farming environment, farm milk consumption, and domestic animal contact would be associated with the low-grade inflammation measured by hsCRP levels in 4.5-year-old children. We evaluated whether relationships exist between children's prenatal and current environmental exposures and current levels of hsCRP and further whether hsCRP levels would be associated with the IgE levels. We also evaluated whether environmental factors play different role in the subclinical inflammation in relation to atopic sensitization. Our hypothesis was to test whether farming environment could induce a systemic low-grade inflammation in 4.5-year-old children, as measured by high-sensitivity CRP levels, thus providing possible protection from allergic disease later in life.

Methods

Study population

The study population consisted of the Protection against Allergy Study in Rural Environments (PASTURE) birth cohort located in rural areas in five European countries: Austria, Finland, France, Germany, and Switzerland (15). Farming and non-farming pregnant women were recruited in the third trimester of pregnancy. Details of this recruitment have been described previously (16). Lifestyle factors and

health issues as well as a broad range of agricultural exposures of the children and mothers were assessed via questionnaires. The total number of study children was 1133 for which data on high-sensitivity CRP levels at the age of 4.5 years were available for 694 (61.3%). Among these, 351 children were living on a farm and 343 in other rural areas at the time of the study.

High-sensitivity C-reactive protein

Venous blood samples were collected from study children at the age of 4.5 years. HsCRP levels were measured by SYNCHRON® System(s) (Beckman Coulter Inc., Fullerton, CA, USA) in Marburg, Germany, according to the manufacturer's instructions (detection limit 0.20 mg/l). The method is based on the highly sensitive Near Infrared Particle Immunoassay rate (17). The measurement seasons were defined as follows, winter: December–February, spring: March–May, summer: June–August and autumn: September–November.

Specific IgE determination

The specific immunoglobulin E (sIgE) of 4.5-year-old study children were analyzed for 19 common allergens, using the Allergy Screen Test Panel for Atopy (Mediawiss Analytic, Moers, Germany) (18). The 13 inhalation allergens tested were two house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), 7 types of pollen (alder, birch, European hazel, grass pollen mixture, rye, mugwort, and plantain), cat, horse, and dog dander, and the mold *Alternaria alternata*. The six food allergens were hen's egg, cow's milk, peanut, hazelnut, carrot, and wheat. Any atopic sensitization was defined as a positive result (sIgE concentration of 0.70 kU/l or greater) to any of the tested allergens, while inhalation or food atopic sensitization was defined as a positive result to any of the 13 inhalation or six food allergens tested, respectively. The following eight inhalation allergens were considered as seasonal: alder, birch, hazel or rye pollen, grass pollen mix, mugwort, plantain, and *Alternaria* species. *Dermatophagoides pteronyssinus* and *farinae*, cat, horse, and dog dander were considered perennial (16). From the total study population of 1133 children, IgE results at 4.5 years were available in 63.2%. Both IgE concentrations and hsCRP values were available for the 652 study children (57.5% of total study population). No significant differences were detected between living environment or IgE levels between those with and without hsCRP results available.

Questionnaires

The first questionnaire was administered to mothers during the third trimester of pregnancy, and follow-up data were collected 2, 12, 18, 24, 36, and 48 months after delivery. The extensive questionnaires focused on respiratory and atopic diseases and farm-related exposures as described earlier (16).

We used data from questionnaires administered during pregnancy and at the time when the children were 4 years old. We focused on exposures earlier identified as major

explanatory variables for the farming effect: time spent in animal sheds and hay barns, consuming farm milk, and having cats or dogs at home (11–14). Maternal current and pre-natal smoking was also included in the analysis.

Data on prenatal and children's exposures to stables and hay barns were created from continuous variables and were categorized into three different groups: Exposure of <15 min per week was regarded as not significant and set to 0; Exposure of between 15 min and <10 h weekly was categorized as 1, while exposures of more than 10 h weekly was

categorized as 2. Consumption of farm milk was also categorized similarly from the continuous variable: Consumption of <10 mL/day was set at 0; Consumption of 10–200 ml daily was grouped as 1; and Consumption of more than 200 ml daily was set to 2.

Body mass index

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. BMI was

Table 1 Associations between hsCRP levels and basic characteristics in 653, 4.5-year-old children

	n	hsCRP (mg/l)*	N (%) of hsCRP ≥ 75th percentile†	aOR (95% CI)‡
Country				
Germany	134	0.11 (0.00/0.77)	34 (25.4)	1
Austria	86	0.10 (0.00/0.65)	19 (22.1)	0.82 (0.42–1.61)
Switzerland	141	0.00 (0.00/0.78)	35 (24.8)	0.93 (0.49–1.75)
France	142	0.35 (0.00/0.90)	42 (29.6)	1.17 (0.65–2.09)
Finland	150	0.00 (0.00/0.58)	33 (22.0)	0.90 (0.43–1.88)
p-Value		0.069	0.608	
Gender				
Male	343	0.00 (0.00/0.57)	68 (19.8)	1
Female	310	0.32 (0.00/0.95)	95 (30.6)	1.84 (1.27–2.66)
p-Value		<0.001	0.002	
BMI (kg/m ²)				
Underweight (<13.4)	25	0.35 (0.00/1.00)	8 (32.0)	1.36 (0.56–3.35)
Healthy weight (13.4–17.1)	429	0.21 (0.00/0.71)	101 (23.5)	1
Overweight (17.2–18.3)	54	0.34 (0.00/1.56)	18 (33.3)	1.66 (0.89–3.11)
Obese or very obese (>18.3)	26	0.80 (0.00/1.20)	14 (53.8)	4.47 (1.94–10.31)
BMI not available	119	0.00 (0.00/0.51)	23 (18.5)	0.83 (0.42–1.68)
p-Value		0.010	0.002	
Place of living at study entry				
Other rural	324	0.23 (0.00/0.73)	78 (24.1)	1
Farm	329	0.21 (0.00/0.76)	85 (25.8)	1.08 (0.74–1.59)
p-Value		0.950	0.651	
Siblings				
No	48	0.00 (0.00/0.57)	9 (18.8)	1
1	234	0.25 (0.00/0.78)	60 (25.6)	1.57 (0.70–3.53)
2	214	0.23 (0.00/0.76)	54 (25.2)	1.37 (0.60–3.13)
3 or more	116	0.22 (0.00/0.66)	28 (24.1)	1.44 (0.59–3.54)
Data missing	41	0.00 (0.00/0.96)	12 (29.3)	2.17 (0.76–6.23)
p-Value		0.746	0.826	
Day care with other children than siblings (at present)				
No	197	0.00 (0.00/0.69)	47 (23.9)	1
With 1–10 children	148	0.00 (0.00/0.79)	39 (26.4)	1.26 (0.75–2.12)
With 11 or more children	308	0.29 (0.00/0.75)	77 (25.0)	0.97 (0.56–1.67)
p-Value		0.069	0.869	
Season of hsCRP measurement				
Winter	110	0.28 (0.00/1.20)	36 (32.7)	1
Spring	167	0.28 (0.00/0.81)	46 (27.5)	0.77 (0.44–1.34)
Summer	157	0.00 (0.00/0.42)	28 (17.8)	0.44 (0.24–0.80)
Autumn	219	0.24 (0.00/0.73)	53 (24.2)	0.68 (0.40–1.15)
p-Value		0.012	0.037	

*hsCRP values are presented as median values with interquartile range. p-Values are estimated by Mann–Whitney and Kruskal–Wallis test.

†p-Values are estimated by Pearson chi-square test.

‡Odds ratios (aOR) and their 95% confidence intervals (CI) are adjusted for parental farming, country, season of measurement, gender, BMI and day care with other children than siblings.

categorized using percentiles based on expert committee recommendations for children: very obese and obese (≥ 95 th percentile), overweight (≥ 85 th to < 95 th percentile), healthy weight (≥ 5 th to < 85 th percentile), and underweight (< 5 th percentile) (19). The percentile definitions were made only for the study children included in the analysis.

Statistical analyses

The hsCRP values below the detection limit were grouped as zero values and included in the analysis. The results were given as median values with interquartile ranges. *p*-Values for the comparisons between different categories in univariate analysis were calculated by the Mann–Whitney *U*-test and the Kruskal–Wallis tests. For multivariate logistic regression analysis, the hsCRP values were dichotomized using the 75th percentile cutoff point (0.75 mg/l). All odds ratios were adjusted for family's farming status at time of study entry and potential confounders, which were at least marginally significantly ($p < 0.10$) associated with hsCRP in the univariate analysis, that is, country, season of sampling hsCRP, gender, BMI, and day care with other children than siblings.

The association between atopic sensitization and hsCRP levels was evaluated as follows: Firstly, the association between any atopic sensitization and hsCRP levels was examined; Secondly, the association between inhaled and food atopic sensitization and the perennial and seasonal inhaled atopic sensitization and hsCRP levels was evaluated separately; Further, the associations between hsCRP levels and environmental factors were evaluated in the sensitized and non-sensitized children separately. All statistical analyses were performed with PASW Statistics 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Of the total sample of 694 children, some children ($n = 41$, 5.9%) had hsCRP values over 5 mg/l and were excluded

from the statistical analyses so as to exclude children with acute infections. Thus, 653 children had hsCRP values equal to or lower than 5.0 mg/l (median: 0.22, interquartile range: 0.00–0.75 mg/l). The hsCRP values were not normally distributed, and 47.9% ($n = 313$) of sample values were lower than the detection limit (< 0.20 mg/l). HsCRP levels were significantly higher among girls and in children who were obese or very obese (Table 1). Values were also significantly lower when measured in summer than in other seasons (medians: 0.00 vs. 0.26 mg/L; $p < 0.001$). In univariate test, French children had significantly higher median hsCRP values compared with other children (medians: 0.35 vs. 0.00 mg/l; $p = 0.003$) and median values also tended to be higher if a child was in a day-care facility with 11 or more children. No associations were detected between values of hsCRP and living on a farm at the time of the study or number of siblings (Table 1).

In the univariate analyses, there was a tendency for higher hsCRP levels in children who had been breastfed for < 2 months duration, but the significance of the association disappeared after multivariate adjustments (Table S1). Further, no significant associations were detected between prenatal, environmental, and farming factors and the levels of hsCRP (Table S1) nor between current maternal smoking, prenatal or current visiting a hay barn and the levels of hsCRP (data not shown) after adjustments. In addition, no significant associations were detected between children who did not drink farm milk in comparison with children who drank either boiled or unboiled farm milk (data not shown).

The prevalence of atopic sensitization against any examined allergen was 45.2% (295/652), and the prevalence of atopic sensitization against any inhalant and against any food allergens was 32.7% (213/652) and 27.3% (178/652), respectively. The prevalence of atopic sensitization against any perennial or seasonal allergens was 21.9% (143/652) and 18.7% (122/652), respectively. There was a decreased risk of sensitization to inhaled allergens and seasonal allergens if a child had hsCRP level lower than the 75th percentile (aOR

Table 2 Prevalence of atopic sensitization and associations with current levels of high-sensitivity C-reactive protein among children aged 4.5 years ($n = 652$)

Type of atopic sensitization	High-sensitivity C-reactive protein						
	<DL		$\geq$ DL and < 75 th percentile		≥ 75 th percentile		p-Value
	Percentage with sensitization	aOR	Percentage with sensitization	aOR (95% CI)	Percentage with sensitization	aOR (95% CI)	
Any	48.9	1	42.6	0.86 (0.58–1.27)	41.1	0.79 (0.52–1.18)	0.478
Inhaled allergens	38.0	1	24.4	0.57 (0.37–0.88)	31.3	0.80 (0.52–1.23)	0.039
Food	28.1	1	30.1	1.17 (0.76–1.79)	22.7	0.78 (0.49–1.24)	0.299
Perennial	24.6	1	15.9	0.63 (0.38–1.05)	23.3	0.93 (0.58–1.52)	0.195
Seasonal	22.4	1	13.6	0.58 (0.35–0.98)	17.2	0.81 (0.49–1.35)	0.122
Total	313		176		163		

DL, Detection limit.

p-Values are obtained from the trend test (Wald) in logistic regression models. Odds ratios are adjusted for country, farmer, season, gender, BMI, and day care with other children than siblings.

0.57, 95% CI 0.37–0.88, and aOR 0.58, 95% CI 0.35–0.98, respectively) compared to levels below the detection limit (Table 2). However, no significant further decrease in risks of different sensitization was observed when hsCRP levels were high, that is, over the 75th percentile (Table 2), or when hsCRP was used as a continuous variable (data not shown). Associations were not changed for perennial and food sensitization even after excluding season of measurement from adjustments (Table S2).

When children were divided into two groups by any atopic sensitization, non-sensitized children who were visiting stables weekly had significantly lower hsCRP levels compared to those who did not spend any time in stables (15 min–10 h aOR 0.40 95% CI 0.16–0.96 and >10 h aOR 0.25 95% CI 0.07–0.90; Table 3). This association was not detected in sensitized children. Further, sensitized children whose mothers had been smoking during the last 2 years had significantly higher hsCRP levels (aOR 2.51, 95% CI 1.12–5.59) and

Table 3 Associations between hsCRP and different environmental and farming factors in relation to any atopic sensitization at the age of 4.5 years

	Non-sensitized				Sensitized			
	n	hsCRP*	N (%) of hsCRP ≥ 75th percentile†	aOR‡ (95% CI)	n	hsCRP*	N (%) of hsCRP ≥ 75th percentile†	aOR‡ (95% CI)
Place of living in last 12 months								
Other rural	177	0.27 (0.00/0.83)	48 (26.7)	1	144	0.00 (0.00/0.54)	30 (20.8)	1
Farm	180	0.24 (0.00/0.81)	48 (27.1)	1.03 (0.61–1.71)	151	0.00 (0.00/0.72)	37 (24.5)	1.20 (0.66–2.20)
p-Value		0.91	1.000	0.925		0.948	0.489	0.551
Duration of breastfeeding (months)								
No breastfeeding	35	0.28 (0.00/0.88)	11 (31.4)	1	20	0.11 (0.00/0.45)	1 (5.0)	1
0.5–2	39	0.36 (0.00/1.24)	14 (35.9)	1.36 (0.49–3.81)	28	0.34 (0.00/0.99)	9 (32.1)	9.44 (1.01–88.55)
2–6	95	0.23 (0.00/0.77)	24 (25.3)	0.84 (0.33–2.12)	73	0.00 (0.00/0.53)	14 (19.2)	4.40 (0.51–38.39)
≥6	188	0.24 (0.00/0.75)	47 (25.0)	0.77 (0.32–1.88)	174	0.00 (0.00/0.73)	43 (24.7)	8.40 (0.98–72.33)
p-Value		0.218	0.485	0.593		0.282	0.116	0.106
Child's visiting a stable weekly in last 12 months								
No (<15 min)	164	0.27 (0.00/0.93)	49 (29.9)	1	118	0.00 (0.00/0.51)	23 (19.5)	1
15 min–10 h	157	0.24 (0.00/0.78)	40 (25.5)	0.40 (0.16–0.96)	149	0.00 (0.00/0.77)	38 (25.5)	1.23 (0.48–3.11)
≥10 h	36	0.00 (0.00/0.63)	7 (19.4)	0.25 (0.07–0.90)	28	0.22 (0.00/0.70)	6 (21.4)	0.80 (0.19–3.36)
p-Value		0.411	0.383	0.072		0.849	0.500	0.674
Child's farm milk consumption (dl/day) in last 12 months								
0	200	0.27 (0.00/0.77)	51 (25.5)	1	154	0.00 (0.00/0.51)	30 (19.5)	1
0–2	65	0.00 (0.00/0.78)	17 (26.2)	1.21 (0.55–2.64)	47	0.00 (0.00/0.95)	15 (31.9)	1.88 (0.77–4.61)
>2	92	0.27 (0.00/0.98)	28 (30.4)	1.48 (0.74–2.96)	94	0.00 (0.00/0.63)	22 (23.4)	1.24 (0.55–2.79)
p-Value		0.584	0.669	0.535		0.916	0.201	0.370
Maternal smoking during pregnancy								
Never smoked	227	0.24 (0.00/0.76)	57 (25.1)	1	182	0.10 (0.00/0.68)	43 (23.6)	1
Not during pregnancy	90	0.34 (0.00/0.97)	28 (31.1)	1.41 (0.79–2.52)	72	0.00 (0.00/0.40)	10 (13.9)	0.52 (0.22–1.20)
During pregnancy	40	0.25 (0.00/0.83)	11 (27.5)	1.24 (0.55–2.79)	41	0.26 (0.00/1.53)	14 (34.1)	1.91 (0.83–4.41)
p-Value		0.272	0.552	0.491		0.179	0.042	0.034
Maternal smoking (during last 2 years)								
No	319	0.26 (0.00/0.83)	87 (27.5)	1	255	0.00 (0.00/0.58)	53 (20.8)	1
Yes	38	0.10 (0.00/0.75)	9 (23.7)	0.94 (0.41–2.17)	40	0.00 (0.00/1.20)	14 (35.0)	2.51 (1.12–5.59)
p-Value		0.637	0.703	0.882		0.694	0.066	0.025
Pets at home (present)								
No cats or dogs	158	0.27 (0.00/0.89)	44 (27.8)	1	125	0.21 (0.00/0.63)	28 (22.4)	1
Only cat/cats	102	0.22 (0.00/0.73)	25 (24.5)	0.83 (0.43–1.61)	75	0.00 (0.00/0.49)	14 (18.7)	0.51 (0.22–1.20)
Only dog/dogs	36	0.27 (0.00/0.97)	12 (33.3)	1.35 (0.58–3.10)	31	0.00 (0.00/1.20)	9 (29.0)	1.70 (0.66–4.35)
Both cat(s) and dog(s)	61	0.26 (0.00/0.75)	15 (24.6)	0.96 (0.44–2.10)	64	0.00 (0.00/0.75)	16 (25.0)	0.83 (0.35–1.98)
p-Value		0.810	0.731	0.773		0.872	0.659	0.194

*HsCRP values are presented as median values with interquartile range, and p-values are estimated by Mann–Whitney or Kruskal–Wallis test.

†p-Values are estimated by Pearson chi-square

‡Odds ratios (aOR) and their 95% confidence intervals (CI) are adjusted for parental farming, country, season of measurement, gender, BMI, and day care with other children than siblings. p-Values are obtained from the trend test (Wald).

moreover, they also tended to have higher levels of hsCRP if the mother had smoked during pregnancy compared to controls (Table 3). No associations were detected between hsCRP values and other determinants, such as place of living at study entry, farm milk consumption, duration of breastfeeding, contact with domestic pets (Table 3), or duration of time spent in hay barns (data not shown).

Discussion

This is first study on the effect of the surrounding environment on low-grade inflammation in children. We observed large differences in levels of hsCRP between seasons of measurement, obesity, and also between genders, but we found only a few associations between farm environmental factors and hsCRP values. Neither did we find strong evidence for an association between low-grade inflammation and the prevalence of atopic sensitization at the age of 4.5 years. No previous studies have evaluated, similarly, the effect of early living environmental factors on concentrations of hsCRP or any other low-inflammatory markers, which makes comparisons with earlier studies difficult. However, our present results confirm those of others reporting an association between higher hsCRP values and female gender, higher BMI and the season of the measurement (1, 5, 20).

We were interested in environmental and farming factors that could possibly induce low-grade inflammation and thereby give protection from allergies; earlier findings have shown that probiotics have the capability to induce such inflammation (9, 10). Unfortunately, we found few clear associations between low-grade inflammation and several characteristics of the farming environment, which have earlier been reported as being associated with protection from allergic disease in childhood (11–14). Among non-sensitized children, those who currently spent more time in stables had significantly lower levels of hsCRP, which is in the opposite of our original hypothesis. It is always possible that more healthy children with lower hsCRP levels spend more time outdoors and in stables compared to children with higher hsCRP levels possible due after recovering recently from infection. We also observed that among sensitized children, maternal current smoking status seemed to be associated with the higher levels of low-grade inflammation at the age of 4.5 years. These results need to be confirmed but they do suggest that some environmental factors may associate with the low-grade inflammation in children, but the risk factors may differ between sensitized and non-sensitized children.

We observed no clear associations between current levels of hsCRP and current sensitization to common allergens. However, there was a suggestion that hsCRP levels that were detectable and yet below the 75th percentile were associated with a decreased risk of atopic sensitization to inhale and seasonal allergens. We believe these results are among the very earliest findings on the associations between hsCRP values and IgE levels in children and should be confirmed by future studies. It should be emphasized that in the present study, both hsCRP and specific IgE levels were measured at the same time, at the age of 4.5 years. Earlier studies have indicated that low-grade intestinal inflammation at early infancy reduces the risk of allergic diseases later (9, 10). Accordingly, there might be a window of opportunity in the early programming of the immune system, and hsCRP values at the age of 4.5 years might be too old already to reflect this.

The strength of this study lies in the prospective cohort nature of the study, which provided large amounts of information on children's prenatal, infant, and childhood environments. All the study children were living in rural areas, which allowed for the comparisons of farmer and non-farmer effects with a wide range of collected data on the early living environment. A weakness of this study was that almost half of the hsCRP values were below the detection limit, which might have reduced the power of the study. As there is a wide seasonal variation in the concentrations of hsCRP, it is possible that a one-time measurement is not sufficiently reliable for analyses of the present kind. However, to reduce the effect of seasonal variation, we adjusted for the season of sampling in the statistical analyses.

In conclusion, we found minor associations between early environmental farming factors and hsCRP levels and further, between hsCRP levels and atopic sensitization in 4.5-year-old children. However, our results suggest that the role played by environmental factor in low-grade inflammation may differ between sensitized and non-sensitized children, although these results need to be confirmed by future studies.

Acknowledgments

We would like to thank the families, all the fieldworkers, and laboratory technicians for enthusiastic collaboration. This study was supported by research grant from the foundation of Ida Montin and by the National Institute for Health and Welfare, Finland.

References

1. Dowd JB, Zajacova A, Aiello AE. Predictors of inflammation in U.S. children aged 3–16 years. *Am J Prev Med*, 2010; **39**: 314–20.
2. Galcheva SV, Iotova VM, Yotov YT, et al. Circulating proinflammatory peptides related to abdominal adiposity and cardiometabolic risk factors in healthy prepubertal children. *Eur J Endocrinol*, 2011; **164**: 553–8.
3. Reinehr T, Stoffel-Wagner B, Roth CL, et al. High-sensitive C-reactive protein, tumor necrosis factor alpha, and cardiovascular risk factors before and after weight loss in obese children. *Metabolism*, 2005; **54**: 1155–61.
4. Giordano P, Del Vecchio GC, Cecinati V, et al. Metabolic, inflammatory, endothelial and haemostatic markers in a group of Italian obese children and adolescents. *Eur J Pediatr*, 2011; **170**: 845–50.
5. MacKenzie KE, Wiltshire EJ, Pena AS, et al. Hs-CRP is associated with weight, BMI, and female sex but not with endothelial function in children with type 1 diabetes. *Pediatr Diabetes*, 2009; **10**: 44–51.
6. Soferman R, Glatstein M, Sivan Y, et al. HsCRP levels: measurement of airway

- inflammation in asthmatic children. *Pediatr Int*, 2008; **50**: 12–6.
7. Michelson PH, Williams LW, Benjamin DK, et al. Obesity, inflammation, and asthma severity in childhood: data from the National Health and Nutrition Examination Survey 2001–2004. *Ann Allergy Asthma Immunol*, 2009; **103**: 381–5.
 8. Snell-Bergeon JK, West NA, Mayer-Davis EJ, et al. Inflammatory markers are increased in youth with type 1 diabetes: the SEARCH Case-Control study. *J Clin Endocrinol Metab*, 2010; **95**: 2868–76.
 9. Marschan E, Kuitunen M, Kukkonen K, et al. Probiotics in infancy induce protective immune profiles that are characteristic for chronic low-grade inflammation. *Clin Exp Allergy*, 2008; **38**: 611–8.
 10. Viljanen M, Pohjavuori E, Haahtela T, et al. Induction of inflammation as a possible mechanism of probiotic effect in atopic eczema-dermatitis syndrome. *J Allergy Clin Immunol*, 2005; **115**: 1254–9.
 11. Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? *J Allergy Clin Immunol*, 2006; **117**: 1374–81.
 12. Riedler J, Braun-Fahrlander C, Eder W, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet*, 2001; **358**: 1129–33.
 13. Von Ehrenstein OS, Von Mutius E, Illi S, et al. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy*, 2000; **30**: 187–93.
 14. Remes ST, Iivanainen K, Koskela H, et al. Which factors explain the lower prevalence of atopy amongst farmers' children? *Clin Exp Allergy*, 2003; **33**: 427–34.
 15. von Mutius E, Schmid S. The PASTURE project: EU support for the improvement of knowledge about risk factors and preventive factors for atopy in Europe. *Allergy*, 2006; **61**: 407–13.
 16. Ege MJ, Herzum I, Buchele G, et al. Prenatal exposure to a farm environment modifies atopic sensitization at birth. *J Allergy Clin Immunol*, 2008; **122**: 407–12, 412 e1–4.
 17. McWhorter VC, Ford LC, Butch AW. Analytical performance of the Synchron LX20 Pro, BN trade mark II and IMMAGE high sensitivity C-reactive protein assays and concordance in cardiovascular risk stratification. *Clin Chim Acta*, 2004; **347**: 71–9.
 18. Herzum I, Blumer N, Kersten W, et al. Diagnostic and analytical performance of a screening panel for allergy. *Clin Chem Lab Med*, 2005; **43**: 963–6.
 19. Barlow SE. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. *Pediatrics*, 2007; **120** (Suppl. 4): S164–92.
 20. Chiriboga DE, Ma Y, Li W, et al. Seasonal and sex variation of high-sensitivity C-reactive protein in healthy adults: a longitudinal study. *Clin Chem*, 2009; **55**: 313–21.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Association of prenatal environmental and farming factors with high-sensitivity C-reactive protein in 653, 4.5-year-old children.

Table S2. Prevalence of atopic sensitization and associations with current levels of high-sensitivity C-reactive protein among children aged 4.5-years (n = 652).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

SUPPLEMENT TABLE 1. Association of prenatal environmental and farming factors with high-sensitivity C-reactive protein in 653 4.5-year-old children

Maternal smoking during pregnancy	n	hsCRP (mg/l)*	N (%) of hsCRP ≥ 75th percentile**	aOR (95% CI)#
Never smoked	410	0.22	100 (24.4)	1
Not during pregnancy	162	0.22	38 (23.5)	0.99 (0.62-1.56)
During pregnancy	81	0.26	25 (30.9)	1.50 (0.85-2.63)
<i>p-value</i>		<i>0.813</i>	<i>0.412</i>	<i>0.331</i>
Maternal visiting a stable weekly during pregnancy (an average)				
No (<15min)	313	0.23	75 (24.0)	1
≥15 min and <10h	171	0.21	44 (25.7)	1.16 (0.67-2.01)
≥ 10 hours	169	0.21	44 (26.0)	1.24 (0.65-2.38)
<i>p-value</i>		<i>0.905</i>	<i>0.850</i>	<i>0.801</i>
Maternal farm milk consumption (dL/day) during pregnancy				
0	358	0.24	85 (23.7)	1
0-2	147	0.00	37 (25.2)	1.32 (0.78-2.22)
> 2	148	0.26	41 (27.7)	1.23 (0.71-2.13)
<i>p-value</i>		<i>0.134</i>	<i>0.644</i>	<i>0.561</i>
Pets at home during pregnancy				
No cats or dogs	274	0.23	63 (23.0)	1
Only cat/cats	161	0.00	45 (28.0)	1.43 (0.87-2.35)
Only dog/dogs	86	0.27	21 (24.4)	1.13 (0.62-2.07)
Both cat(s) and dog(s)	132	0.23	34 (25.8)	1.36 (0.76-2.44)
<i>p-value</i>		<i>0.976</i>	<i>0.707</i>	<i>0.530</i>
Duration of breastfeeding (months)				
No breastfeeding	55	0.23	12 (21.8)	1
0.5-2	67	0.36	23 (34.3)	1.95 (0.83-4.59)
2-6	168	0.10	38 (22.6)	1.21 (0.55-2.65)
≥6	363	0.10	90 (24.8)	1.41 (0.65-3.02)
<i>p-value</i>		<i>0.051</i>	<i>0.270</i>	<i>0.383</i>
Child's visiting a stable weekly				
No (<15 min)	282	0.25	72 (25.5)	1
≥15 min and <10 hours	307	0.21	78 (25.4)	0.64 (0.34-1.19)
≥ 10 hours	64	0.11	13 (20.3)	0.43 (0.17-1.09)
<i>p-value</i>		<i>0.795</i>	<i>0.664</i>	<i>0.193</i>
Child's farm milk consumption (dL/day)				
0	355	0.23	81 (22.8)	1
0-2	112	0.00	32 (28.6)	1.46 (0.82-2.59)
> 2	186	0.22	50 (26.9)	1.32 (0.79-2.22)
<i>p-value</i>		<i>0.851</i>	<i>0.365</i>	<i>0.388</i>

* Median values of hsCRP and p-values estimated by Kruskal-Wallis test

** P-values are estimated by Pearson Chi-square

Odds ratios (aOR) and their 95% confidence intervals (CI) are adjusted for parental farming, country, season of measurement, gender, BMI and day care with other children than siblings. P-values are obtained from the trend test (Wald).

SUPPLEMENT TABLE 2. Prevalence of atopic sensitization and associations with current levels of high-sensitivity C-reactive protein among children aged 4.5-years (n=652)

	High Sensitivity C-reactive protein						
	< DL		≥ DL and < 75th percentile		≥ 75th percentile		
Type of atopic sensitization	% with sensitization	aOR	% with sensitization	aOR(95% CI)	% with sensitization	aOR(95% CI)	P-value
Any	48.9	1	42.6	0.85 (0.58-1.25)	41.1	0.80 (0.54-1.20)	0.498
Inhaled allergens	38.0	1	24.4	0.57 (0.37-0.87)	31.3	0.78 (0.51-1.20)	0.033
Food	28.1	1	30.1	1.18 (0.77-1.80)	22.7	0.82 (0.52-1.30)	0.365
Perennial	24.6	1	15.9	0.62 (0.38-1.02)	23.3	0.95 (0.59-1.54)	0.161
Seasonal	22.4	1	13.6	0.57 (0.34-0.96)	17.2	0.77 (0.46-1.27)	0.098
Total	313		176		163		
DL= Detection limit							

P-values are obtained from the trend test (Wald) in logistic regression models. Odds ratios are adjusted for country, farmer, gender, BMI and day care with other children than siblings

SUPPLEMENT TABLE 1. Association of prenatal environmental and farming factors with high-sensitivity C-reactive protein in 653 4.5-year-old children

Maternal smoking during pregnancy	n	hsCRP (mg/l)*	N (%) of hsCRP ≥ 75th percentile**	aOR (95% CI)#
Never smoked	410	0.22	100 (24.4)	1
Not during pregnancy	162	0.22	38 (23.5)	0.99 (0.62-1.56)
During pregnancy	81	0.26	25 (30.9)	1.50 (0.85-2.63)
<i>p-value</i>		0.813	0.412	0.331
Maternal visiting a stable weekly during pregnancy (an average)				
No (<15min)	313	0.23	75 (24.0)	1
≥15 min and <10h	171	0.21	44 (25.7)	1.16 (0.67-2.01)
≥ 10 hours	169	0.21	44 (26.0)	1.24 (0.65-2.38)
<i>p-value</i>		0.905	0.850	0.801
Maternal farm milk consumption (dL/day) during pregnancy				
0	358	0.24	85 (23.7)	1
0-2	147	0.00	37 (25.2)	1.32 (0.78-2.22)
> 2	148	0.26	41 (27.7)	1.23 (0.71-2.13)
<i>p-value</i>		0.134	0.644	0.561
Pets at home during pregnancy				
No cats or dogs	274	0.23	63 (23.0)	1
Only cat/cats	161	0.00	45 (28.0)	1.43 (0.87-2.35)
Only dog/dogs	86	0.27	21 (24.4)	1.13 (0.62-2.07)
Both cat(s) and dog(s)	132	0.23	34 (25.8)	1.36 (0.76-2.44)
<i>p-value</i>		0.976	0.707	0.530
Duration of breastfeeding (months)				
No breastfeeding	55	0.23	12 (21.8)	1
0.5-2	67	0.36	23 (34.3)	1.95 (0.83-4.59)
2-6	168	0.10	38 (22.6)	1.21 (0.55-2.65)
≥6	363	0.10	90 (24.8)	1.41 (0.65-3.02)
<i>p-value</i>		0.051	0.270	0.383
Child's visiting a stable weekly				
No (<15 min)	282	0.25	72 (25.5)	1

≥15 min and <10 hours	307	0.21	78 (25.4)	0.64 (0.34-1.19)
≥ 10 hours	64	0.11	13 (20.3)	0.43 (0.17-1.09)
<i>p-value</i>		<i>0.795</i>	<i>0.664</i>	<i>0.193</i>

Child's farm milk consumption (dL/day)

0	355	0.23	81 (22.8)	1
0-2	112	0.00	32 (28.6)	1.46 (0.82-2.59)
> 2	186	0.22	50 (26.9)	1.32 (0.79-2.22)
<i>p-value</i>		<i>0.851</i>	<i>0.365</i>	<i>0.388</i>

* Median values of hsCRP and p-values estimated by Kruskal-Wallis test

** P-values are estimated by Pearson Chi-square

Odds ratios (aOR) and their 95% confidence intervals (CI) are adjusted for parental farming, country, season of measurement, gender, BMI and day care with other children than siblings. P-values are obtained from the trend test (Wald).

SUPPLEMENT TABLE 2. Prevalence of atopic sensitization and associations with current levels of high-sensitivity C-reactive protein among children aged 4.5-years (n=652)

	High Sensitivity C-reactive protein						
	< DL		≥ DL and < 75th percentile		≥ 75th percentile		
Type of atopic sensitization	% with sensitization	aOR	% with sensitization	aOR(95% CI)	% with sensitization	aOR(95% CI)	P-value
Any	48.9	1	42.6	0.85 (0.58-1.25)	41.1	0.80 (0.54-1.20)	0.498
Inhaled allergens	38.0	1	24.4	0.57 (0.37-0.87)	31.3	0.78 (0.51-1.20)	0.033
Food	28.1	1	30.1	1.18 (0.77-1.80)	22.7	0.82 (0.52-1.30)	0.365
Perennial	24.6	1	15.9	0.62 (0.38-1.02)	23.3	0.95 (0.59-1.54)	0.161
Seasonal	22.4	1	13.6	0.57 (0.34-0.96)	17.2	0.77 (0.46-1.27)	0.098
Total	313		176		163		
DL= Detection limit							

P-values are obtained from the trend test (Wald) in logistic regression models. Odds ratios are adjusted for country, farmer, gender, BMI and day care with other children than siblings