

ORIGINAL ARTICLE

Childhood overweight and asthma symptoms, the role of pro-inflammatory proteins

M. B. M. Bekkers^{1,2}, B. Brunekreef^{1,3}, J. C. de Jongste⁴, M. Kerkhof⁵, H. A. Smit³, D. S. Postma⁶, U. Gehring¹ and A. H. Wijga²

¹Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands, ²Centre for Prevention and Health Services Research, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, ³Julius Centre for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands, ⁴Department of Paediatrics, Erasmus University Medical Center, Sophia Children's Hospital, Rotterdam, The Netherlands, ⁵Department of Epidemiology and Bioinformatics, University of Groningen, Groningen, The Netherlands and ⁶Department of Pulmonology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

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Summary

Background Systemic inflammation is suggested as a mechanism by which overweight might induce asthma. However, few studies have linked childhood overweight, inflammation and asthma.

Objective To study the association between body mass index (BMI), asthma symptoms and pro-inflammatory proteins.

Methods High-sensitivity C-reactive protein (hs-CRP), complement factor 3 (C3) and 4 (C4) concentrations, and body weight and height were available for 359 4-year-old children participating in the Prevention and Incidence of Asthma and Mite Allergy birth cohort study. Data on asthma symptoms were obtained by yearly questionnaires. Logistic regression and generalized estimating equations were used to analyse the cross-sectional and prospective associations between BMI, asthma symptoms and pro-inflammatory proteins.

Results BMI was associated with asthma symptoms {odds ratio [OR] 1.43 [95% confidence interval (CI): 1.08–1.88] per BMI standard deviation scores [SDS]}. The inclusion of hs-CRP, C3 and C4 in the statistical models did not change this association. C3 was cross-sectionally associated with frequent asthma symptoms [OR per interquartile range of C3: 1.97 (95% CI: 1.20–3.24)] and prospectively with asthma symptoms [OR: 1.48 (95% CI: 1.04–2.09)], independent of BMI SDS.

Conclusions and Clinical Relevance We showed no evidence for a role of hs-CRP, C3 and C4 in the association between BMI and asthma symptoms. C3 concentrations were associated with (frequent) asthma symptoms, independent of BMI.

Keywords asthma, body mass index standard deviation score, complement factor 3, complement factor 4, high-sensitivity C-reactive protein, inflammation

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Correspondence:

Marga Bekkers, Centre for Prevention and Health Services Research (pb 101), National Institute for Public Health and the Environment (RIVM), PO Box 1, 3720 BA Bilthoven, The Netherlands.
E-mail: marga.bekkers@rivm.nl

Introduction

Childhood asthma and overweight have reached high prevalence levels, each having a considerable public health impact [1, 2]. Observational studies, including our own, have shown an increased risk of asthma in overweight adults [3] and children [4–8]. Suggested mechanisms for this relation include a sedentary lifestyle, changes in immune function, insulin resistance and airway narrowing [9–12]. Additionally, systemic inflammation is suggested as a mechanism by which overweight might

induce asthma [10, 11]. Previous studies showed increased serum concentrations of the pro-inflammatory proteins high-sensitivity C reactive protein (hs-CRP) [13–22], complement factor 3 (C3) [18, 23–27] and complement factor 4 (C4) [18, 24, 27] with higher body mass index (BMI) in adults and adolescents. Few studies looked specifically at young children; Skinner et al. [28] found that obese children (age 3–5 years) were at an increased risk of elevated (> 1 mg/L) hs-CRP concentrations, and Cianflone et al. [29] showed increased C3 concentrations with increasing percentage of ideal body weight in

2–6-year-old children. Adipose tissue secretes C3 and activates the subsequent pathway [30]. Furthermore, adults and children with asthma have higher hs-CRP concentrations compared with non-asthmatic individuals [14, 16, 17, 31–33]. The complement system is involved in the aetiology of asthma [34, 35]. These studies did not investigate whether the pro-inflammatory proteins mediate the association between BMI and asthma. To our knowledge, only one, recent study linked overweight, asthma and inflammation in children, using CRP as a marker of inflammation [16].

We studied the association between BMI, the pro-inflammatory proteins and asthma symptoms in four steps. In the first step, we studied whether BMI measured at age 4 was associated with asthma symptoms measured at age 4 and with asthma symptoms at age 5–8. In the second, we studied the association between BMI and the pro-inflammatory proteins. In the third step, we studied the association between the pro-inflammatory proteins and asthma symptoms. In the last step, we studied the role of the pro-inflammatory proteins in the association between BMI and asthma symptoms.

Methods

Study design and study population

The children in this study participated in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study and were born in the Netherlands in 1996–1997. A detailed description of the study design has been published previously [36]. Mothers were recruited from the general population during pregnancy visiting one of 52 prenatal clinics. These prenatal clinics were spread over the Netherlands and were a representative sample of prenatal clinics. At baseline, the cohort consisted of 4146 pregnant women, of whom 183 (5%) were lost to follow-up before any data of the child had been collected (Fig. 1). Therefore, the study started with 3963 newborns. Postal questionnaires were sent to the parents during pregnancy, at the child's ages of 3 and 12 months, and yearly thereafter up to the age of 8 years. At the age of 4 years, only a selected subsample ($n = 1808$) of the PIAMA population was invited for a medical examination, i.e. all children of allergic mothers who were still in the study and a random sample of children of non-allergic mothers [36]. Only a limited number of the children participating in the medical examination ($n = 1288$) were willing to provide a blood sample ($n = 750$). For a group of children for whom aliquots were still left after IgE analysis, serum samples were analysed for pro-inflammatory proteins. Analyses were successful for 376 samples collected from children when they were 4 years old (see Fig. 1 for details).

All children ($n = 11$) with hs-CRP concentrations >10 mg/L reported fever at the day of the medical examination or in the last 2 weeks before the medical examination or having a cold at the day of the medical examination. Therefore, these high levels of hs-CRP were considered to be due to the acute-phase response and these children were excluded from all analyses. Six children without information on either body weight or height or asthma symptoms were excluded from all analyses. Finally, the population under study consisted of 359 children. The study protocol was approved by the medical ethics committees of the participating institutes and all parents gave written informed consent.

Outcome assessment

In the yearly questionnaires, parents reported whether their child had an attack of wheeze and/or an episode of dyspnoea and/or a prescription of inhaled corticosteroids for respiratory or lung problems by a medical doctor in the preceding year. These questions were partly based on the International Study of Asthma and Allergies in Childhood core questionnaire [37]. In the PIAMA study, 'asthma symptoms' at ages 3–8 years are defined as at least one attack of wheeze and/or at least one episode of dyspnoea and/or a prescription of inhaled corticosteroids in the last 12 months. 'Frequent asthma symptoms' are defined as having asthma symptoms and ≥ 4 attacks of wheeze or ≥ 4 attacks of dyspnoea in the last 12 months. Children with asthma symptoms and children with frequent asthma symptoms were compared with children with no asthma symptoms.

In the collected blood samples, specific IgE was determined on the following common inhalant allergens: house dust mite (HDM), cats, dogs, grass (*Dactylis glomerata*), birch pollen and *Alternaria alternata*. Allergic asthma symptoms were defined as having asthma symptoms as well as a specific IgE concentration of ≥ 0.70 IU/mL to at least one of the inhalant allergens. Children with allergic or non-allergic asthma symptoms were compared with children with no asthma symptoms.

Anthropometric measures

Anthropometric measurements were performed by professionals during the medical examination. Body weight, wearing only underwear, was measured to the nearest 0.1 kg and height (cm) was measured to the nearest millimetre. BMI was calculated as body weight in kilograms divided by height squared in metres (kg/m^2). Standard deviation scores (SDS) of BMI for age and sex were calculated using the reference growth curves of the Dutch Fourth nationwide Growth Study [38].

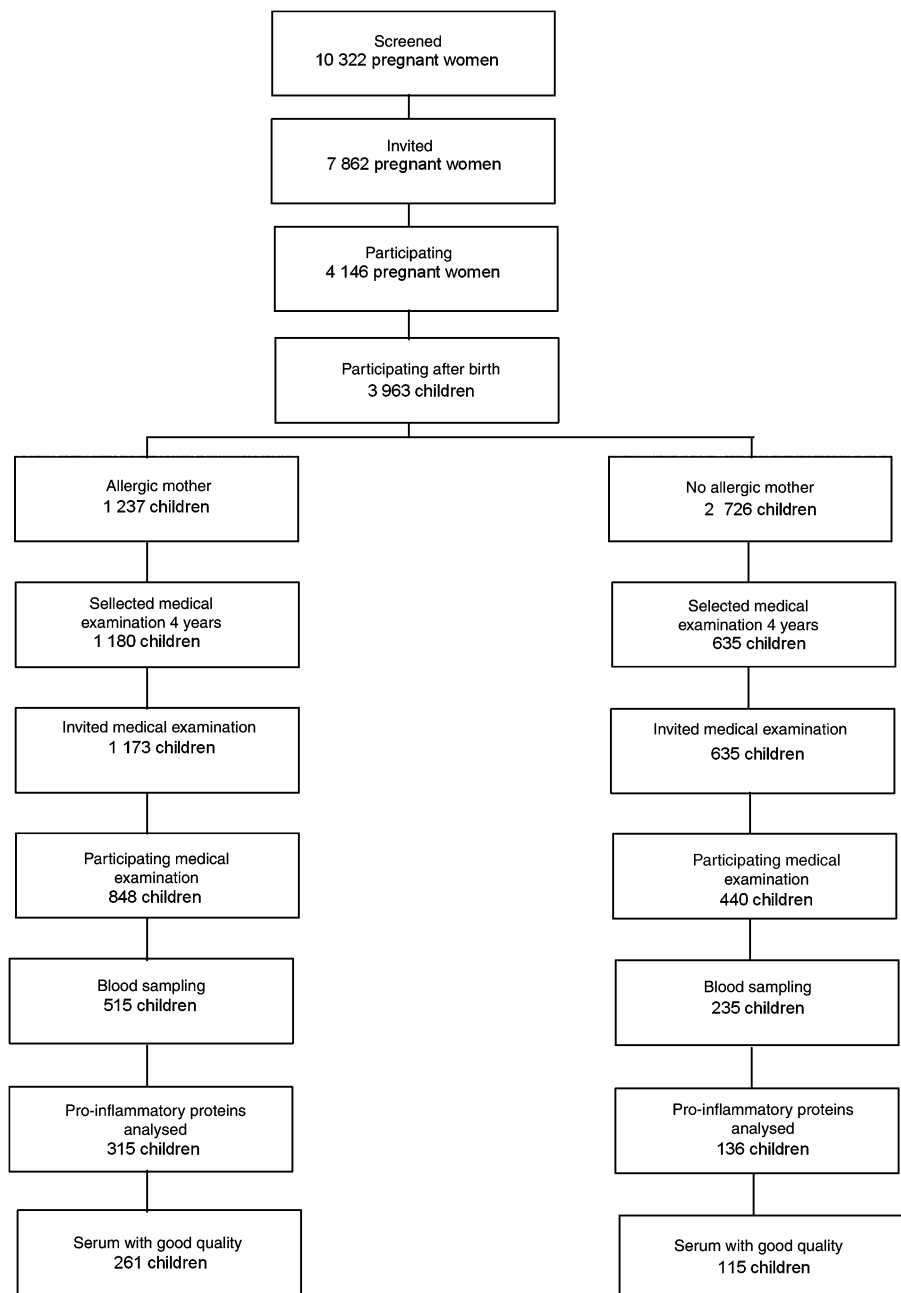


Fig. 1. Recruitment scheme of children in the Prevention and Incidence of Asthma and Mite Allergy birth cohort study.

Assessment of pro-inflammatory proteins

Pro-inflammatory protein concentrations were determined in non-fasting serum samples (Synchro LX20-Pro, Beckman Coulter, Woerden, the Netherlands), using an immuno-turbidimetric assessment. These measurements are not affected by fasting/non-fasting states [39]. The assay could detect a minimal hs-CRP concentration of 0.2 mg/L and values below this level were classified as below the detection limit. Minimal concentrations of 100 mg/L C3 and 50 mg/L C4 could be detected.

Assessment of effect modifiers and confounders

Maternal allergy was reported in a validated questionnaire during pregnancy and defined as current allergy to HDM or pets, or ever asthma, or current hayfever [40]. In the questionnaires, the parents reported maternal education, maternal smoking during pregnancy, smoking in the house by anyone when the child was 4 years old and gender. Maternal education was measured as the highest education completed and then divided into three categories (low, intermediate and high education). Data on

TV-hours per day were collected by a yearly questionnaire and for the first time in the 5-year questionnaire.

Statistical analyses

In the first step, we studied the association between BMI SDS and asthma symptoms cross-sectionally and prospectively. The cross-sectional associations between BMI SDS measured at age 4 and the prevalence of asthma symptoms at age 4 were studied using logistic regression. The prospective associations between BMI SDS measured at age 4 and asthma symptoms at the ages 5–8 years were studied using generalized estimating equations (GEE), taking into account the serial correlations between repeated measurements in the same individual. As correlations between repeated measurements can be expected to depend on the time lag between two measurements, a three-dependent correlation matrix was modelled. The interaction of BMI SDS with age was included in the GEE model, allowing associations to vary with age.

Gender, age, maternal allergy, maternal education, maternal smoking during pregnancy, smoking by anyone in the house when the child was 4 years old and TV-hours, indicators of physical inactivity, were considered as potential confounders. Effect modification by maternal allergy, maternal education, gender and allergic sensitization was tested.

In addition, allergic and non-allergic asthma symptoms were analysed separately in association with BMI SDS. To study the possibility of a U-shaped association between BMI and the outcome variables, the association between BMI SDS quintiles and the asthma outcomes was assessed.

Secondly, we studied the associations between BMI SDS and the pro-inflammatory proteins, both measured at age 4 years, in linear regression models.

In the third step, we studied the associations of hs-CRP, C3 and C4 measured at age 4 with asthma symptoms measured at age 4 in logistic regression models. We used GEE models to study the prospective associations between the pro-inflammatory proteins and asthma symptoms at age 5–8 years. The lowest hs-CRP group (reference, $n = 117$) consisted of all children with an hs-CRP concentration below the detection limit. The children with an hs-CRP concentration above the detection limit were distributed equally in the two other groups (hs-CRP concentrations 0.20–0.59 mg/L and hs-CRP concentrations 0.61–9.89). No children had an hs-CRP concentration of 0.60 mg/L. C3 and C4 were included in the model as continuous variables and effect estimates were calculated per interquartile range (IQR) (C3: IQR = 266.3 mg/L; C4: IQR = 71.6 mg/L).

Three different models were used to study these associations: a crude model, a model with adjustment for potential confounders and a model with additional adjustment for BMI SDS.

Finally, in the fourth step, we added the pro-inflammatory proteins to the statistical models of BMI SDS and asthma symptoms to observe whether the associations between BMI SDS and asthma symptoms were attenuated.

Data analysis was conducted using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Eighteen percent of the children ($n = 65$) were overweight (including obesity) according to the International Obesity Task Force standards [41] and 26.0% ($n = 91$) of the children had asthma symptoms at age 4 (Table 1). The parents of the children in our analyses were more highly educated (39.7%) compared with the original PIAMA population (35.0%). Furthermore, the children in our analyses differed from the original PIAMA population in the prevalence of maternal allergy. 68.5% of the children in our analyses had a mother with allergy, whereas in the original PIAMA population, 31.2% of the children had an allergic mother. This is related to decisions made about

Table 1. General characteristics of the 4-year-old study population, n (%)

General characteristics	4-year-old children ($n = 359$)	
	n	%
Girls	152	42.3
Maternal allergy	246	68.5
Maternal education		
Low	61	17.2
Intermediate	153	43.1
High	141	39.7
Maternal smoking during pregnancy	54	15.1
Asthma symptoms	91	26.0
Frequent asthma symptoms	30	10.5
Wheeze	59	17.0
Dyspnoea	61	17.4
Corticosteroid medication use	39	11.4
Allergic asthma symptoms	26	7.2
Overweight (including obesity)*	65	18.2
Obesity*	15	4.2
Hs-CRP concentration below detection limit	117	32.6
	Mean	SD
BMI (kg/m ²)	16.4	1.37
BMI SDS	0.54	0.91
Hs-CRP concentration mg/L [†]	0.78	1.36
C3 concentration g/L	1.43	0.21
C4 concentration g/L	0.22	0.07

*Defined according to the International Obesity Task Force standards [41].

[†]Geometric mean (geometric standard deviation) of the children with an hs-CRP concentration > detection limit (0.2 mg/L).

BMI, body mass index; SDS, standard deviation score; hs-CRP, high-sensitivity C-reactive protein; C3 and C4, complement factor 3 and 4.

which subgroups to invite for medical examination at age 4 as explained in the 'Methods'.

Body mass index standard deviation scores and asthma symptoms

In the first step; at age 4, BMI SDS was significantly associated with the presence of asthma symptoms in the last 12 months; odds ratio (OR), 1.43; [95% confidence interval (95%CI), 1.08–1.88] per BMI SDS and with frequent asthma symptoms (OR, 1.74; 95%CI, 1.12–2.71) per BMI SDS (Table 2). The association between BMI SDS and asthma outcomes was linear; no U-shaped association was detected.

Adjustment for maternal allergy, maternal education and maternal smoking during pregnancy did not change this association. Effect modification by gender, maternal allergy, maternal education and allergic sensitization was not shown as interaction terms with gender, maternal allergy and allergic sensitization were not significant. Hours spent watching TV did not confound the association between BMI SDS and asthma.

Associations between BMI SDS at age 4 and asthma outcomes at ages 5–8 were also positive but failed to reach statistical significance (Table 2).

The cross-sectional association between BMI SDS and non-allergic asthma symptoms was stronger (OR, 1.62; 95%CI, 1.16–2.26) than the association between BMI SDS and allergic asthma symptoms (OR, 1.08; 95%CI, 0.70–1.69).

Body mass index standard deviation scores and pro-inflammatory proteins

Second, we studied whether BMI SDS was associated with increased concentrations of the pro-inflammatory proteins. No clear association of BMI SDS and the pro-inflammatory proteins was shown in linear regression

analyses; hs-CRP: $\beta = 0.01$, $P = 0.96$; C3: $\beta = -1.29$, $P = 0.91$ and C4: $\beta = -3.65$, $P = 0.34$.

Pro-inflammatory proteins and asthma

In the third step, we studied whether the pro-inflammatory proteins were associated with asthma symptoms. The correlation (r) between hs-CRP and C3 and C4 was 0.27 and 0.37, respectively, and r was 0.46 for the correlation between C3 and C4. Therefore, we performed logistic regression analyses with separate models for hs-CRP, C3 and C4 each. C3, but not hs-CRP and C4, was consistently associated with an increased risk of (frequent) asthma symptoms both cross-sectionally and prospectively and independent of BMI SDS (Table 3). C4 was significantly associated with frequent asthma symptoms only prospectively and after adjustment for BMI [OR, 1.42; (95%CI, 1.00–2.01)] (Table 3). The associations between hs-CRP 0.20–0.59 mg/L and asthma symptoms tended to be negative. Hs-CRP concentrations from 0.61 to 9.89 mg/L were consistently, but not significantly, associated with wheeze, and cross-sectionally also with dyspnoea (Table 3). Overall, no clear association between hs-CRP concentrations and asthma symptoms was observed.

We repeated the analyses with the pro-inflammatory proteins together in one model. The effect estimates of C3 and C4 did not change; those of hs-CRP did. Possibly, this is due to multi-collinearity.

The associations of allergic asthma with hs-CRP, C3 and C4 were positive [hs-CRP 0.20–0.59 mg/L: 1.35 (95%CI 0.48–3.83), hs-CRP 0.61–9.89 mg/L: 1.58 (95%CI 0.57–4.37), C3: 1.68 (95%CI 1.00–2.82), C4: 1.26 (95%CI 0.86–1.84)] and were stronger than the associations between the pro-inflammatory proteins and non-allergic asthma [hs-CRP 0.20–0.59 mg/L: 1.08 (95%CI 0.55–2.15), hs-CRP 0.61–9.89 mg/L: 1.10 (95%CI 0.55–2.15), C3: 1.16 (95%CI 0.82–1.65), C4: 0.87 (95%CI 0.63–1.19)], but not significant.

Table 2. Associations [OR (95%CI)] between BMI SDS and asthma symptoms, frequent asthma symptoms, wheeze, dyspnoea and use of corticosteroids, $n = 359$

	Asthma symptoms	Frequent asthma symptoms	Wheeze	Dyspnoea	Corticosteroid use
Cross-sectional association between BMI SDS (age 4) and asthma symptoms (age 4)					
BMI SDS*	1.43 (1.08–1.88)	1.75 (1.13–2.71)	1.18 (0.86–1.61)	1.53 (1.11–2.10)	1.51 (1.03–2.21)
Prospective † association between BMI SDS (age 4) and asthma symptoms (age 5–8)					
BMI SDS*	1.15 (0.88–1.52)	1.37 (0.91–2.07)	1.31 (0.87–1.96)	1.26 (0.92–1.74)	1.25 (0.86–1.83)

*Model adjusted for maternal allergy, maternal education and maternal smoking during pregnancy.

†The presented OR is the overall OR from the general estimating equations model.

Bold indicates statistical significant association $P < 0.05$.

Odds ratio per 1 SDS increase in BMI.

Asthma symptoms: ≥ 1 attack of wheeze and/or ≥ 1 episode of dyspnoea and/or a prescription of inhaled corticosteroids in the last 12 months (reference: no asthma symptoms); frequent asthma symptoms: having asthma symptoms and ≥ 4 attacks of wheeze or ≥ 4 attacks of dyspnoea in the last 12 months (reference: no asthma symptoms).

BMI SDS, body mass index standard deviation score.

Table 3. Associations [OR (95%CI)] between hs-CRP, C3 and C4 asthma symptoms, frequent asthma symptoms, wheeze, dyspnoea and use of corticosteroids, *n* = 359

	Asthma symptoms	Frequent asthma symptoms	Wheeze	Dyspnoea	Corticosteroid use
Cross-sectional association between the pro-inflammatory proteins (age 4) and asthma symptoms (age 4)					
Hs-CRP 0.20–0.59 mg/L*	1.11 (0.61–2.04)	1.11 (0.43–2.89)	1.56 (0.76–3.20)	0.90 (0.44–1.85)	0.81 (0.34–1.93)
Hs-CRP 0.61–9.89 mg/L*	1.19 (0.65–2.19)	1.35 (0.53–3.46)	1.43 (0.68–2.98)	1.51 (0.77–2.98)	1.24 (0.54–2.83)
C3*	1.30 (0.95–1.78)	1.97 (1.20–3.24)	1.58 (1.10–2.28)	1.38 (0.96–1.96)	1.90 (1.23–2.93)
C4*	0.99 (0.76–1.29)	1.21 (0.83–1.77)	1.18 (0.87–1.59)	0.98 (0.72–1.32)	1.18 (0.83–1.67)
Hs-CRP 0.20–0.59 mg/L†	0.94 (0.50–1.75)	0.86 (0.32–2.32)	1.42 (0.68–2.97)	0.75 (0.36–1.58)	0.67 (0.28–1.64)
Hs-CRP 0.61–9.89 mg/L†	1.13 (0.61–2.10)	1.24 (0.48–3.24)	1.40 (0.67–2.93)	1.42 (0.71–2.84)	1.13 (0.49–2.63)
C3†	1.33 (0.96–1.82)	1.92 (1.16–3.19)	1.64 (1.13–2.37)	1.37 (0.95–1.96)	1.91 (1.23–2.95)
C4†	1.03 (0.79–1.35)	1.27 (0.86–1.88)	1.22 (0.90–1.66)	1.00 (0.73–1.36)	1.23 (0.86–1.76)
Prospective association between the pro-inflammatory proteins (age 4) and asthma symptoms (age 5–8)					
Hs-CRP 0.20–0.59 mg/L*	0.82 (0.42–1.61)	0.72 (0.27–1.94)	0.66 (0.24–1.77)	0.74 (0.35–1.58)	0.87 (0.34–2.24)
Hs-CRP 0.61–9.89 mg/L*	1.01 (0.53–1.94)	0.84 (0.33–2.13)	1.46 (0.62–3.42)	0.85 (0.41–1.76)	1.02 (0.41–2.52)
C3*	1.48 (1.04–2.09)	1.18 (0.74–1.86)	1.65 (1.04–2.61)	1.38 (0.93–2.05)	1.84 (1.15–2.94)
C4*	1.26 (0.96–1.66)	1.39 (0.98–1.97)	1.31 (0.97–1.76)	1.31 (0.97–1.76)	1.44 (1.03–2.04)
Hs-CRP 0.20–0.59 mg/L†	0.80 (0.41–1.59)	0.64 (0.22–1.81)	0.63 (0.33–1.73)	0.71 (0.33–1.54)	0.82 (0.31–2.12)
Hs-CRP 0.61–9.89 mg/L†	0.99 (0.52–1.91)	0.79 (0.31–2.01)	1.42 (0.60–3.35)	0.84 (0.41–1.73)	0.98 (0.39–2.43)
C3†	1.47 (1.04–2.07)	1.16 (0.73–1.83)	1.64 (1.04–2.60)	1.37 (0.92–2.04)	1.83 (1.14–1.94)
C4†	1.27 (0.97–1.67)	1.42 (1.00–2.01)	1.32 (0.98–1.78)	1.32 (0.98–1.78)	1.47 (1.04–2.07)

*Model adjusted for maternal allergy, maternal education and maternal smoking during pregnancy.

†Model additionally adjusted for BMI SDS.

Bold indicates statistical significant association $P < 0.05$.

Asthma symptoms: ≥ 1 attack of wheeze and/or ≥ 1 episode of dyspnoea and/or a prescription of inhaled corticosteroids in the last 12 months (reference: no asthma symptoms); Frequent asthma symptoms: having asthma symptoms and ≥ 4 attacks of wheeze or ≥ 4 attacks of dyspnoea in the last 12 months (reference: no asthma symptoms).

The reference category for hs-CRP was the group of children with hs-CRP concentrations below the detection limit. Results of C3 and C4 are displayed per interquartile range. C3 and C4, complement factor 3 and 4; Hs-CRP, high-sensitivity C-reactive protein.

Table 4. Associations [OR (95%CI)] between BMI SDS and asthma symptoms, frequent asthma symptoms, wheeze, dyspnoea and use of corticosteroids, additionally adjusted for the pro-inflammatory proteins, *n* = 359

	Asthma symptoms	Frequent asthma symptoms	Wheeze	Dyspnoea	Corticosteroid use
Cross-sectional association between BMI SDS (age 4) and asthma symptoms (age 4)					
BMI SDS*	1.43 (1.08–1.91)	1.71 (1.10–2.65)	1.17 (0.85–1.60)	1.57 (1.13–2.18)	1.57 (1.07–2.31)
Prospective association between BMI SDS (age 4) and asthma symptoms (age 5–8)					
BMI SDS*	1.21 (0.92–1.59)	1.45 (0.97–2.16)	1.41 (0.94–2.12)	1.36 (0.99–1.88)	1.30 (0.92–1.84)

*Model adjusted for maternal allergy, maternal education, maternal smoking during pregnancy, hs-CRP, C3, C4.

Bold indicates statistical significant association $P < 0.05$.

Asthma symptoms: ≥ 1 attack of wheeze and/or ≥ 1 episode of dyspnoea and/or a prescription of inhaled corticosteroids in the last 12 months (reference: no asthma symptoms); Frequent asthma symptoms: having asthma symptoms and ≥ 4 attacks of wheeze or ≥ 4 attacks of dyspnoea in the last 12 months (reference: no asthma symptoms).

Odds ratio per 1 SDS increase in BMI.

BMI SDS, body mass index standard deviation score.

Pro-inflammatory proteins in the association between body mass index standard deviation scores and asthma symptoms

The inclusion of hs-CRP, C3 and C4 concentrations in the regression models did not attenuate the cross-sectional association between BMI SDS and asthma symptoms or the prospective association between BMI SDS at age 4 and asthma symptoms in the following years (i.e. 5–8) (see Table 4 in comparison with Table 2). This was also the case for the cross-sectional and prospective associations

between BMI SDS and frequent asthma symptoms, wheeze, dyspnoea and the use of corticosteroids (Table 4).

Discussion

We found a significant cross-sectional association between BMI SDS and asthma symptoms in 4-year-old children that was not attenuated by adjustment for pro-inflammatory markers. This suggests that the association between BMI SDS and asthma symptoms was not

explained by these pro-inflammatory proteins in this age group. We also found, for the first time, that, independent of BMI SDS, C3 was associated with asthma symptom prevalence in young children.

We were able to study the association between BMI SDS and asthma symptoms in a prospective birth cohort, using body weight and height measured by trained investigators. Information on asthma symptoms and the use of corticosteroids was available for each year, and in addition, we measured sensitization. In contrast to previous studies, we were able to study the role of C3 and C4 in addition to hs-CRP, and were able to adjust for potential confounders like maternal smoking and maternal allergy during pregnancy, and maternal education reported at age 1 year of the child.

The definition of asthma symptoms in the PIAMA birth cohort study was based on wheeze, dyspnoea and prescription of inhaled corticosteroids reported by the parents. Although these factors are important indicators for asthma in children, wheeze and dyspnoea are also common symptoms of respiratory infections in children and some misclassification cannot be excluded. Therefore, we also used the definition of frequent asthma symptoms, which is more stringent by only including children who, in addition to having asthma symptoms according to our definition, also had four or more attacks of wheeze or dyspnoea in the past 12 months. A limitation of this study was that serum pro-inflammatory protein concentrations were only available for a subset of children. This resulted in differences between the children in our study and the original PIAMA birth cohort with regard to maternal educational level and percentage of children with an allergic mother. Because effect modification by these variables was not present, we assume that selective serum sample availability has not affected the generalizability of our findings. The prevalence of obesity was only 4%; this is representative of the 4-year-old population in the Netherlands [42]. Besides, systemic inflammation may be more marked in obese compared with overweight children; however, already in overweight children, inflammation processes are going on [43].

Recently, in line with our observations, Michelson et al. [16] showed, in a relatively young population (<20 years old), that high BMI SDS was cross-sectionally associated with an increased risk of asthma. They additionally found that children with increased CRP levels were at a higher risk of having asthma, whereas we did not find an association between hs-CRP concentrations and asthma symptoms. The associations between BMI SDS and asthma and between CRP levels and asthma found in their study were independent of each other. Unfortunately, they did not report the association between BMI SDS and asthma without adjustment for CRP levels; therefore, no conclusions on attenuation of the association between BMI SDS and asthma after adjustment for CRP can be drawn. The association between CRP levels and asthma they found

was specifically in the severe asthmatics, which may explain our different findings, as we were not able to differentiate between three degrees of severity of asthma.

In contrast to previous studies reporting higher hs-CRP concentrations in overweight [14, 19–21, 28] and asthmatic [14, 32, 33] children, we did not find associations between hs-CRP and BMI and asthma. The children in the present study were young compared with the previous studies; possibly, a longer period of overweight is needed to cause systemic inflammation [28]. C4 has not been studied in children before. Two previous studies, one in adults [25] and one in children, showed higher C3 concentrations with increasing BMI or waist circumference, whereas we did not show increased C3 concentrations with increasing BMI SDS. This association may only be visible at a later stage in life or only in the very obese. On the basis of experimental models of asthma in animals, it is well established that the complement system plays a role in the pathophysiology of asthma [34, 35]. In contrast to previous studies in adults showing an association of hs-CRP with non-allergic asthma, and not with allergic asthma [13, 17], we did not observe associations between hs-CRP and allergic asthma symptoms and non-allergic asthma symptoms.

Some studies suggest that the impact of overweight on asthma is partly mediated by (chronic) systemic inflammation [10, 11]. Our data do not support this for 4-year-old children as the associations we observed between BMI SDS and asthma outcomes were completely insensitive to adjustment for the inflammation markers we used in the present study. This implies that the association between BMI and asthma may be explained by pro-inflammatory markers other than those used in the present study, or as mentioned before, that a longer period of being overweight is needed to cause systemic inflammation; possibly, the effect of systemic inflammation is only present at a later age. In this context, Skinner et al. [28] found that at the age of 3–5 years only being very obese was associated with an increased risk of abnormal CRP values, whereas in 15–17-year-old children, this association was also found in overweight children.

In conclusion, the associations between BMI SDS and asthma in this study were not affected by adjustment for the inflammation markers hs-CRP, C3 and C4. C3 concentrations were associated with (frequent) asthma symptoms, independent of BMI SDS.

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