The role of IgG and IgE in the development of allergy and asthma
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Do levels of immunoglobulin antibodies to foods predict the development of immunoglobulin E antibodies to cat, dog and/or mite?

P.E.D. Eysink, P.J.E. Bindels, S.O. Stapel, B.J.A.M. Bottema, J.S. Van der Zee, R.C. Aalberse

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Abstract

**Background** In children at high risk of inhalation allergy, food sensitization is associated with an increased risk for sensitization to inhalant allergens. Furthermore, this association was also found in a cross-sectional study.

**Objective** To examine in a prospective study, whether levels of IgG to foods (i.e. mixture of wheat and rice, mixture of soybean and peanut, egg white, cow's milk, meat, orange and potato) indicate an increased risk for the future development of IgE antibodies to inhalant allergens in a low-risk population and whether they can be used as predictors of the subsequent development of IgE antibodies in young, initially IgE negative children.

**Methods** Coughing children, aged 1-5, visiting their GPs, were tested for IgE antibodies to mite, dog and cat (RAST) and IgG (ELISA) to foods. All IgE negative children were retested for IgE antibodies after two years. The IgG results (66-percentiles) of the first blood sample were compared to the RAST-scores of the second blood sample.

**Results** After two years, 51 of 397 (12.8%) originally IgE negative children, had become IgE positive for cat, dog and/or mite. An increased IgG antibody level to wheat-rice (OR = 2.2) and to orange (OR = 2.0) indicated an increased risk of developing IgE to cat, dog or mite allergens. In addition to IgG to mixture of wheat-rice and orange; total IgE, breastfeeding, eczema as a baby and age were the most important predictors for the subsequent development of IgE to inhalant allergens.

**Discussion** An increased IgG antibody level to mixture of wheat-rice or orange, indicates an increased risk of developing IgE to cat, dog or mite allergens. This indicates that excessive activity of the mucosal immune system is present before IgE antibodies to airborne allergens can be demonstrated. Nevertheless, IgG to foods is not very helpful (with a positive predictive value of 16.5%, and negative predictive value of 90.6%) in identifying individual children at risk in clinical practice. However, besides other risk factors, IgG to wheat-rice and to orange could be useful as a screening test for studies in the early identification, i.e. before IgE antibodies can be de-
ected, of children with an increased risk of developing IgE antibodies in the future.

Introduction

Cross-sectional [1] and prospective [2] studies strongly indicate that exposure to allergens during a critical period early in life, may influence the development of both IgE antibodies and allergic diseases later in life [3]. Various environmental factors have been thought to enhance or protect against the development of both IgE antibodies and allergic disease [4]. Calkhoven et al. [5] found that IgG to foods is associated with an increased future risk for sensitization to inhalant allergens. However, this study was performed in a high-risk group of children and comprised many children with atopic eczema. When the results were corrected for eczema, statistical significance did not persist. Several other studies [6-10] reported that atopic children have higher levels of IgG subclass, particularly IgG4, antibodies to milk and egg than non-atopic children. However, the children in these studies were at a high risk for the development of specific IgE (either they had a parental history of atopic disease [6,7] and/or they had eczema (or asthma) themselves [9-11]). In an earlier, cross-sectional, study [12], we found that the levels of IgG to a panel of foods were associated with the presence of IgE to common allergens in a group of low-medium risk young children. However, only longitudinal prospective studies can determine whether high IgG antibody levels to foods predict the subsequent development of IgE to inhalant allergens. As this was a cross-sectional study, the question if IgG to foods might be useful as an early marker for the development of IgE-mediated allergy remains open.

Three possible explanations have been suggested as to why IgG to foods is associated with IgE to inhalants: (1) a mucosal defect in the gut which results in an increased permeability to macromolecules [13], (2) enhanced immu-
nological hyperreactivity [14,15] and, (3) immunological cross-reactivity of inhalant-allergens with food-antigens [16]. Therefore, immunological priming (e.g. IgG antibody responses) towards foods could predispose to the development of inhalant allergy.

The aim of the present study was to confirm the observation that high levels of IgG to foods predict the future development of IgE-mediated allergy in children even when not at high risk. In other words: can IgG antibodies to foods measured in the first blood sample, be used to predict the outcome: IgE antibodies to airborne allergens in the second sample (two years later)? We therefore evaluated in a prospective, longitudinal study whether IgG to foods can be used as an early biomarker for the development of IgE antibodies in young, initially IgE negative children.

**Subjects and Methods**

**Selection of the study population**

In this study, 136 general practitioners from the north-western and centre part of The Netherlands (urban and rural areas) participated. The general practitioners included all 1-5 year old children with coughing complaints lasting for more than five days, who visited the general practice from February 1995 to January 1997. Informed consent was obtained from the parents of the children.

On entrance, the parents completed a questionnaire with 11 questions on coughing, asthma and allergy in the family, breastfeeding, eczema as a baby, contact with pets and bedroom floor covering.

**Blood samples**

On entrance to the study, a blood sample was collected by blotting three drops of capillary blood from the children’s fingers on filter paper for the determination of total IgE and specific IgE for house dust mite, cat and dog. Children scoring >0.2 RU/ml on one or more of the allergens were ex-
cluded from further participation in the study. All children with a blood sample representing ≥10 μl plasma and scoring less than 0.2 RU/ml on the three allergens were also tested for their IgG antibody response to a panel of selected foods. The foods tested were cow’s milk, chicken’s egg white, orange, meat, potato, a mixture of wheat and rice and a mixture of soybean and peanut. These foods were selected in an earlier study [17]. The selected antigens cover the normal spectrum of foods given to infants in The Netherlands.

After a two year follow-up, the originally IgE negative children (n = 530) were invited into the general practitioner’s office for a second blood sample. Again, blood was spotted on filter paper and tested for total IgE and specific IgE for house dust mite, cat and dog.

**Laboratory methods**

*Determination of total IgE and specific IgE*

A convenient method for obtaining blood from young children was used: essentially three drops of blood, obtained by fingerprick, were adsorbed on filter paper (Schleicher & Schuell 2294, K120258). Blood samples were eluted from the filter paper using 1 ml of PBS/0.05% [v/v] Tween-20. Assays for measuring total serum IgE and specific IgE were adjusted for application of small amounts of plasma. Total IgE was measured essentially as described earlier [18]; specific IgE directed against house dust mite, cat and dog dander was determined by RAST as described by Aalberse et al. [16], with slight modifications. Total IgE results were expressed in international units per millilitre (IU/ml), RAST results were expressed in RAST units per millilitre (RU/ml, based on a calibration curve of chimeric IgE antibody [19], one RAST unit represents approximately 2.4 ng of specific IgE). All test results were corrected for actual amounts of plasma used in the tests. The amount of plasma per eluate was determined on the basis of the albumin content assuming that plasma corresponds with 42 g/l. Human serum albumin in
eluted plasma was assayed using a nephelometric method (BN³), Behring Werke, Freiburg, Germany.

_Determination of food-specific IgG antibodies_

Measurement of food-specific IgG antibodies in blood sample eluates was performed by ELISA, essentially as previously described [12]. Foods were obtained from local food stores, and homogenized at 10% (w/v), essentially according to Björksten et al. [20], with minor modifications. Briefly, orange and potato were extracted in 0.1 mol/l phosphate buffer pH 7.5, containing 2% polyvinylpolypyrrolidone, 7 mmol/l diethylithiocarbamate, 2 mmol/l ethylenediaminetetraacetic acid disodium salt (EDTA) and 2.6 mmol/l NaN₃. Peanut, soy and rice were extracted in water at pH 8.0. Wheat flour was extracted in water and in 0.5 mol/l NaCl; extracts were mixed afterwards. Pork was homogenized in water, containing 2.6 mmol/l NaN₃. After stirring for 1 hour particulates were removed by centrifugation at 18.000 g for 30 min. Extracts were defatted, dialyzed against distilled water and lyophilized. Cow's milk and egg white: pH of skimmed milk was adjusted to 4.8 with acetic acid, resulting in precipitation of the casein fraction. The casein precipitate was resuspended in 0.9% NaCl, pH 8.5. The whey supernatant and the resuspended casein were stirred for 1 h, centrifuged, dialyzed against distilled water and lyophilized. After separation from the yolk, egg white was diluted 1/10 with saline. After stirring, centrifugation and dialysis, the preparation was lyophilized.

For the ELISAs, 1 µg of food extract per well was coated to Maxisorp (Nunc, Denmark) ELISA plates, and 0.1 µl of plasma was used per test (all blood samples were diluted until they contained 42 µg of albumin/ml). 100 µl of serum sample was added to each well. Optical density was read at 450 nm in an automatic ELISA reader (Bio-Tek Instruments, Winooski, VT, USA). On every ELISA plate tested, a number of wells were coated with α-gliadin, and these wells were incubated with twofold dilutions of a gliadin-positive reference serum, attributed with 100 arbitrary units per millilitre (AU/ml) of gliadin-specific IgG, serving as a calibration curve. Therefore, it was possible
to express the ELISA results, obtained with different serum samples for food-specific IgG, as AU/ml. All tests were performed in duplicate. Because some sera showed some non-specific IgG binding to the plate material, all sera were tested in a parallel ELISA procedure in which no food was coated, and the results were corrected for this non-specific binding. Some blood samples did not (after IgE testing) contain sufficient amounts of plasma for testing all food antigens. In those cases the following test-sequence of the foods was used: orange, milk, egg, mixture of soybean-peanut, mixture of wheat-rice, potato and meat. In all blood samples IgG to orange and milk was tested. The samples without results for the remaining foods (nine missing values for potato, two for egg, five for wheat-rice, two for soybean-peanut, eight for meat), received a modus-value of the concerned food.

**Data analysis**

The results of the RAST were dichotomized as IgE negative or IgE positive. Children were considered IgE negative if they scored <0.5 RU/ml for all three allergens in the second RAST.

The results of the food assays were dichotomized high or low using the 66-percentile of the total group as a cut-off value (P66). The 66-percentile was chosen because for all foods the lower boundaries of the 95% confidence interval around the 66-percentile [21] were higher than the detection limit of 2.0 AU/ml.

Logistic regression analysis was used to assess the independent effects (odds ratios (ORs) with 95% confidence intervals) of IgG to foods on becoming IgE positive, adjusted for family history of allergy, breastfeeding, eczema as a baby, age and total IgE in the first blood sample. Total IgE was not normally distributed and therefore logtransformed before entering into the logistic regression model. Likelihood ratio statistics were used as a criterion for selection in the logistic regression model. In all analyses, a $P$-value <0.05 was regarded as statistically significant.
Data analysis was performed with SPSS 9.0 for Windows.

Results

General characteristics
From February 1995 to January 1997, 654 eligible children were tested. However, 126 (19.3%) of them scored >0.20 RU/ml for cat, dog and/or house dust mite. These children were excluded from the present analyses. Due to technical reasons or because there was not enough eluate available, 31 of the remaining 528 eluates were not used for IgG-food-analyses. Thus, in the blood samples of 497 children food-specific IgG antibodies were measured.

After 24 months 419 children participated in the second IgE test: five children had a blood sample that was too small for IgE analysis. As to the remaining 414 children, food-specific IgG measures in the first blood sample were available for 397 of them. Thus, for 397 children both food-specific IgG measures in the first blood sample and IgE results in the second blood sample were available. No statistical significant differences were found in age, gender, and food-specific IgG antibodies between the 397 children with both a first and a second blood sample and children with only a first blood sample (n = 100).

The general characteristics of the 397 children with all IgE and all IgG measures in the study population are presented in Table 4.1. Of these children, 51 (12.8%) had become IgE positive (≥0.50 IU/ml for one or more allergens) during the two year follow-up. The children who had become IgE positive during the two year follow-up were somewhat younger than the children who were still IgE negative, but this was not statistically significant (P-value = 0.21).

Of the 51 children who had become IgE positive, 36 (70.6%) had one positive RAST score, 11 (21.6%) had two positive RAST scores and 4 (7.8%) children were IgE positive for cat, dog and mite.
Table 4.1  General characteristics of the children in the study population \((n = 397)\)

<table>
<thead>
<tr>
<th></th>
<th>IgE positive(^1)</th>
<th>IgE negative(^2)</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>total number (%)</td>
<td>51 (12.8)</td>
<td>346 (87.2)</td>
<td>397 (100.0)</td>
</tr>
<tr>
<td>gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>boys</td>
<td>29 (56.9)</td>
<td>178 (51.4)</td>
<td>207 (52.1)</td>
</tr>
<tr>
<td>girls</td>
<td>22 (43.1)</td>
<td>168 (48.6)</td>
<td>190 (47.9)</td>
</tr>
<tr>
<td>age at the time of the 1(^{st}) RAST (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19 (37.3)</td>
<td>138 (39.9)</td>
<td>157 (39.5)</td>
</tr>
<tr>
<td>2</td>
<td>21 (41.2)</td>
<td>91 (26.3)</td>
<td>112 (28.2)</td>
</tr>
<tr>
<td>3</td>
<td>5 (9.8)</td>
<td>63 (18.2)</td>
<td>68 (17.1)</td>
</tr>
<tr>
<td>4</td>
<td>6 (11.8)</td>
<td>54 (15.6)</td>
<td>60 (15.1)</td>
</tr>
<tr>
<td>mean age at the time of the 1(^{st}) RAST (months) (± sd)</td>
<td>28.3 (± 12.9)</td>
<td>30.1 (± 13.8)</td>
<td>29.8 (± 13.7)</td>
</tr>
<tr>
<td>age at the time of the 2(^{nd}) RAST (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15 (29.4)</td>
<td>77 (22.3)</td>
<td>92 (23.2)</td>
</tr>
<tr>
<td>4</td>
<td>15 (29.4)</td>
<td>89 (25.7)</td>
<td>104 (26.2)</td>
</tr>
<tr>
<td>5</td>
<td>11 (21.6)</td>
<td>87 (25.1)</td>
<td>98 (24.7)</td>
</tr>
<tr>
<td>6</td>
<td>7 (13.7)</td>
<td>62 (17.9)</td>
<td>69 (17.4)</td>
</tr>
<tr>
<td>7</td>
<td>3 (5.9)</td>
<td>31 (9.0)</td>
<td>34 (8.6)</td>
</tr>
<tr>
<td>mean age at the time of the 2(^{nd}) RAST (months) (± sd)</td>
<td>58.5 (± 14.6)</td>
<td>61.3 (± 15.4)</td>
<td>61.0 (± 15.3)</td>
</tr>
<tr>
<td>number of positive RAST scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0 (0)</td>
<td>346 (100.0)</td>
<td>346 (87.2)</td>
</tr>
<tr>
<td>1</td>
<td>36 (70.6)</td>
<td>0 (0)</td>
<td>36 (9.1)</td>
</tr>
<tr>
<td>2</td>
<td>11 (21.6)</td>
<td>0 (0)</td>
<td>11 (2.8)</td>
</tr>
<tr>
<td>3</td>
<td>4 (7.8)</td>
<td>0 (0)</td>
<td>4 (1.0)</td>
</tr>
<tr>
<td>total IgE (95% CI) of 1(^{st}) blood sample (IU/ml)</td>
<td>34.7 (2.3-529.0)</td>
<td>12.2 (0.7-223.3)</td>
<td>14.0 (0.7-269.6)</td>
</tr>
<tr>
<td>total IgE (95% CI) of 2(^{nd}) blood sample (IU/ml)</td>
<td>109.9 (6.0-2025.0)</td>
<td>25.4 (1.5-426.5)</td>
<td>34.7 (1.5-603.7)</td>
</tr>
</tbody>
</table>

Data expressed as numbers (percentages), means (± sd) or geometric means (95% CI)

1. IgE positive (≥0.5 RU/ml) at the second RAST
2. IgE negative (<0.5 RU/ml) at the second RAST
The children had predominantly become IgE positive for mites (40/51: 15 of the 19 IgE positive one year olds, 16 of the 21 IgE positive two year olds, all five IgE positive three year olds and four of the five IgE positive four year olds).

In Figure 4.1 the cumulative frequency of the food-specific IgG antibody assays in the first blood sample for the study population are shown. In all children food-specific IgG antibodies to at least four foods could be measured. The range in IgG to foods varied per food, e.g. orange ranged from <2.0 to 60.5 AU/ml, while milk ranged from 3.3 to 1259.5 AU/ml. IgG to most foods did not change with age, except milk, which decreased with age, and egg, which increased with age (Figure 4.2).

Figure 4.1 Cumulative frequency of IgG to foods (AU/ml) in first blood sample for the study population (n = 397). Curve of IgG to soybean-peanut is not displayed because this curve is similar to the curve of IgG to egg.
Logistic regression analyses

Logistic regression analyses with increased IgG antibody levels of the foods (66-percentiles) were carried out to find predictors for developing IgE to inhalants. Mixture of wheat-rice (OR = 2.2 (95% CI = 1.2-3.9)) and orange (OR = 2.0 (95% CI = 1.1-3.7)) were the most important (significant) predictors of becoming IgE positive after two years (Table 4.2). Because IgG to milk and IgG to egg changed with age, we also performed logistic regression analyses adjusted for age at the time of the first blood sample. The ORs did not change with age in the model (data not shown). When a multivariate logistic regression was performed with all foods in the model, both mixture of wheat-rice and orange were borderline significant (Table 4.2). Again, age did not influence the results.
Table 4.2  Development of IgE (in second blood sample) in relation to IgG to foods in first blood sample; odds ratios (with 95% CI) (n = 397)

<table>
<thead>
<tr>
<th>P66 food</th>
<th>IgE positive</th>
<th>IgE positive</th>
<th>IgE positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>potato</td>
<td>1.4 (0.7-2.5)</td>
<td>1.1 (0.5-2.2)</td>
<td>1.3 (0.7-2.4)</td>
</tr>
<tr>
<td>milk</td>
<td>1.4 (0.8-2.6)</td>
<td>1.2 (0.6-2.2)</td>
<td>1.4 (0.7-2.6)</td>
</tr>
<tr>
<td>egg</td>
<td>1.0 (0.5-1.8)</td>
<td>0.7 (0.4-1.4)</td>
<td>1.0 (0.5-1.9)</td>
</tr>
<tr>
<td>orange</td>
<td>2.0 (1.1-3.7)*</td>
<td>1.8 (0.9-3.6)</td>
<td>1.9 (1.0-3.5)*</td>
</tr>
<tr>
<td>mixture of soybean-peanut</td>
<td>1.6 (0.9-2.9)</td>
<td>1.4 (0.7-2.7)</td>
<td>1.5 (0.8-2.8)</td>
</tr>
<tr>
<td>mixture of wheat-rice</td>
<td>2.2 (1.2-3.9)*</td>
<td>1.8 (1.0-3.8)</td>
<td>2.0 (1.1-3.6)*</td>
</tr>
<tr>
<td>meat</td>
<td>0.9 (0.5-1.8)</td>
<td>0.7 (0.3-1.4)</td>
<td>0.9 (0.5-1.7)</td>
</tr>
</tbody>
</table>

1  univariate analyses
2  multivariate analyses
3  logistic regression analyses per food adjusted for age at the time of the first blood sample, eczema as a baby, breastfeeding and family history of allergy

* P < 0.05

On the basis of known risk factors (such as family history of allergy, breastfeeding, eczema as a baby, age and total IgE in the first blood sample) and the 66-percentile of wheat-rice or of orange, we wanted to predict which children were to become IgE positive after two years. An increased IgG antibody level of mixture of wheat-rice and orange (adjusted for family history, breastfeeding, total IgE in first blood sample, age at the time of the first blood sample, and eczema) were most important predictors of becoming IgE positive to inhalants (Table 4.2). Of these predictors, an increased IgG antibody level of mixture of wheat-rice and orange, breastfeeding, higher total IgE in first blood sample, age and having had eczema as a baby were the most important ones for the subsequent development of IgE to inhalant-allergens (Table 4.3). For the multivariate analyses, the ORs for wheat-rice are shown, the results for orange are similar (data not shown).
Table 4.3  Development of IgE (in second blood sample) in relation to IgG to foods (in first blood sample) adjusted for total IgE, breastfeeding, family history of allergy, eczema and age, odds ratios (with 95% CI), (n = 397)

<table>
<thead>
<tr>
<th></th>
<th>IgE positive$^1$</th>
<th>IgE positive$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>total IgE$^1$</td>
<td>3.5 (1.8-5.1)*</td>
<td>3.2 (1.9-5.3)*</td>
</tr>
<tr>
<td>age at the time of 1$^{st}$ RAST (1-2 years vs. 3-4 years)</td>
<td>0.4 (0.2-0.9)*</td>
<td>0.4 (0.2-0.9)*</td>
</tr>
<tr>
<td>positive family history of allergy</td>
<td>1.3 (0.7-2.5)</td>
<td></td>
</tr>
<tr>
<td>breastfeeding</td>
<td>2.7 (1.2-6.3)*</td>
<td>2.9 (1.3-6.6)*</td>
</tr>
<tr>
<td>having had eczema as a baby</td>
<td>1.9 (0.9-4.0)</td>
<td>2.0 (1.0-4.0)</td>
</tr>
<tr>
<td>orange</td>
<td>1.4 (0.7-2.9)</td>
<td></td>
</tr>
<tr>
<td>mixture of wheat &amp; rice</td>
<td>1.6 (0.8-3.1)</td>
<td>1.8 (1.0-3.5)</td>
</tr>
</tbody>
</table>

1 logistic regression analyses with orange, wheat-rice, total IgE, age at the time of the first blood sample, eczema as a baby, breastfeeding and family history of allergy in the model and being IgE positive (≥0.5 RU/ml) at the second RAST as the dependent variable
2 logistic regression analyses with wheat-rice, total IgE, age at the time of the first blood sample, eczema as a baby, and breastfeeding in the model and being IgE positive (≥0.5 RU/ml) at the second RAST as the dependent variable
3 total IgE was logtransformed before entering into the logistic regression model
* $P < 0.05$

Discussion

In this study, we have investigated the (longitudinal) relation between IgG to foods (i.e. mixture of wheat and rice, mixture of soybean and peanut, egg white, cow’s milk, meat, orange and potato) in originally IgE negative children and the subsequent development of IgE antibodies to inhalant allergens (cat, dog and/or house dust mite). Increased IgG antibody levels to wheat-rice and to orange were most important in predicting the subsequent development of IgE antibodies to cat, dog and/or mite. Furthermore, in breastfed children and in children having had eczema as a baby, an increased IgG antibody level to wheat-rice and to orange indicates an increased risk of developing IgE to cat, dog, and mite allergens in all age groups.
All foods selected for logistic regression analyses were dichotomized high or low using the 66-percentile as a cut-off value. A lower cut-off, e.g. the median, in most cases leads to a less robust classification. The reason is that a large number of sera score close to this lower cut-off value and therefore retesting would have a high probability of resulting in a change in classification. Higher cut-off values, like the 75-percentile, were less discriminating than the 66-percentile as a cut-off value, which led to a decreased sensitivity and specificity.

After two years of follow-up, 419 out of 528 originally IgE negative children participated. The children who did not participate for the second test did not differ in age, gender, and food-specific IgG antibodies from the children who participated in the second test.

The coughing children who had become IgE positive during the two year follow-up were somewhat younger at the time of the RASTs than the children who were still IgE negative, but this was not statistically significant. This could mean that children who will become IgE positive in the future, have coughing complaints at a younger age than the children who will stay IgE negative. Coughing and becoming IgE positive seem to be more strongly associated in younger children than in older children. This is in contrast with asthma-like complaints, e.g. wheezing, in which wheezing seems to be associated with allergy at a later age [22-24].

Almost a quarter of the children was younger than four years of age at the time of the second RAST. As reported in other studies [6,25], few children will become sensitized to airborne allergens during the first three years of life. Therefore, the younger children in our study had less chance of becoming IgE positive. So, if all the IgE negative three and four year olds had had their RAST at five or six years of age, some of them would also have become IgE positive to inhalant-allergens. This could mean that some potentially IgE positive children are now classified as IgE negative and this will have influenced our results. This might underestimate the predictive relationships with food antigens.
In all children, IgG antibodies to foods could be measured. IgG antibody levels to potato, orange, wheat-rice, meat and soybean-peanut did not change with age. The IgG antibody responses to egg increased with age and responses to milk decreased with age from one year onwards. These findings are in accordance with previous cross-sectional [26] and prospective [6,8,27] studies, showing that egg-specific IgG reaches a peak around three to four years of age and IgG antibodies to milk start to decrease from 6 to 18 months onwards.

In contrast to the findings in this study, all food-specific IgGs were associated with IgE in a previous, cross-sectional, study [12]. However, in that study the children were one year of age and most of the atopic children were IgE positive for egg and milk. In the current study, we only measured IgE to inhalant allergens and not IgE to egg and milk. Furthermore, the previous study was a cross-sectional study and the present study is a longitudinal study. This could partly explain why all foods were associated with IgE in the previous study. Nevertheless, we expected to find an association between IgG to egg and the subsequent development of IgE to inhalant allergens, because IgG to egg and IgE to egg are closely associated [28,29]. Moreover, in several studies [30-34], it was found that IgE antibodies to egg at the age of one year were predictive of subsequent sensitization to inhalant allergens at the age of three. In our study, however, we could not confirm this observation, probably because of the small group of children that became IgE positive among the one year olds.

Most strongly associated with the development of IgE to inhalants were increased IgG levels of wheat-rice and orange. It is not likely that the tested foods and tested inhalants share common epitopes. Therefore, the third possible explanation for the association between IgG to foods and IgE to inhalants mentioned in the introduction, i.e. immunological cross-reactivity, is not supported. The starting point of our study was that cross-reactivity between food antigens and airborne allergens, e.g. between grass pollen and wheat, might result in cross-reactive priming. In the earlier study of
Calkhoven et al. [5], the measurement of IgE to grass pollen was included and the predictive value of elevated levels of IgG to foods was found to be similar for grass pollen and for mite. However, no cross-reactivity between wheat and mite can be demonstrated. The two other explanations, i.e. mucosal defects or enhanced immunological hyperreactivity, are still valid and plausible possibilities, because hyperactivity of the immune system (either reacting sooner or slowed down less fast, or both) is also present in tetanus [14] and *Helix pomatia* hemocyanin [15].

The ability to predict which children will become allergic is very important. Several predictors have been proposed, e.g. elevated titres of IgE in cord blood and elevated serum levels of IL-4, but they have not yet shown to be useful as screening tests for the prediction of atopy. At present only family history appears to have some useful predictive value in the development of asthma and atopic disease [35]. Most children have IgG antibodies to foods and these IgG antibodies can often be detected before IgE antibodies to inhalants [6,10]. Furthermore, in this study we found that increased levels of IgG to wheat-rice and to orange are associated with an increased risk of developing IgE antibodies in the future. Nevertheless, IgG to foods are probably not very useful as a screening test in individual children, mainly because of their low specificity (53.0%) and low positive predictive value (16.5%). However, with respect to the results of this study, IgG antibodies to wheat-rice and to orange could be useful as markers for the (early) identification of at-risk children in studies.

In conclusion, increased IgG antibody levels of mixture of wheat-rice and orange, breastfeeding, total IgE and having had eczema as a baby were the most important predictors for subsequent development of IgE to inhalant-allergens. An increased IgG antibody level to wheat-rice indicates an independent increased risk of developing IgE to cat, dog or mite allergens in all age groups. This indicates that an excessive activity of the mucosal immune system is present before IgE antibodies to airborne allergens can be demonstrated. This might reflect either a deficiency of the mucosal barrier or an immunological hyperactivity. Nevertheless, IgG to foods is not very helpful
in identifying individual children at risk in clinical practice. However, besides family history and breastfeeding, IgG to wheat-rice or IgG to orange could be useful as a screening test for future prospective studies, in the early identification, i.e. before IgE antibodies can be detected, of children with an increased risk of developing IgE antibodies in the future.

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