

Relation between IgG antibodies to foods and IgE antibodies to milk, egg, cat, dog and/or mite in a cross-sectional study

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Summary

Background Because IgG antibodies to foods can be detected before IgE antibodies to inhalants, increased levels of IgG antibodies to foods might be used as a predictor of IgE-mediated allergy in initially nonatopic children.

Objective To examine the cross-sectional 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) and specific IgE to cat, dog, mite, milk and egg white in 1-year-old children.

Methods All atopic children ($n = 120$; 58 with and 62 without eczema) and a random sample of the nonatopic children ($n = 144$) of the Bokaal study were tested on their IgG response to foods. The IgG results of the food assays were dichotomized high or low using the 66th centile as a cut-off value.

Results Atopic children more often had high IgG levels to foods than nonatopic children. IgG to egg white ($OR = 7.50$) and mixture of wheat and rice ($OR = 4.79$) were most strongly associated with positive specific IgE. In a stepwise logistic regression analysis egg white, mixture of wheat and rice, and orange were selected ($OR = 3.76$, $OR = 2.43$, and $OR = 2.11$, respectively). In children without eczema higher levels of IgG to foods were still significantly associated with atopy, which was most prominent for egg white, orange and cow's milk.

Conclusion An increased IgG antibody level to foods, especially to egg white, orange, and mixture of wheat and rice, indicates an increased risk of having IgE to cat, dog, mite, egg and/or milk allergens, even in the noneczematous group. Therefore, in another prospective study we are currently investigating the usefulness of IgG in early identification, i.e. before IgE antibodies can be detected, of children with an increased risk of developing allergic diseases in the future.

Keywords: Bokaal study, atopic disease, foods, IgE, IgG, inhalants, predictor

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Introduction

Various environmental factors have been thought to influence the development of both IgE antibodies and allergic disease. Exposure to inhaled allergen is important, but other

environmental factors are also of influence such as passive smoking [1–3], diet of mother and child perinatally [4–6], pets [3,7,8], number of siblings [3,9,10], breast-feeding [8,11,12], and month of birth [13,14].

In allergic diseases, IgE is regarded as the most important immunoglobulin, but IgG antibodies have also been reported as being associated with allergic diseases [15–18]. Calkhoven *et al.* [19] found that food sensitization is associated with an

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increased future risk for sensitization to inhalant allergens. However, this study was performed in a relatively high-risk group of children and comprised many children with atopic eczema. The results suggested that IgG might be useful in determining a child's risk of becoming allergic within a few years and therefore could be used as an early marker for the development of IgE-mediated allergy.

With respect to the future development of IgE-mediated allergy, it is important to assess the association between IgG to foods and IgE to inhalant allergens in a low-medium risk population. To examine this association, we studied 264 children, not selected for allergy risk, at the age of 1 year (from the Bokaal study). The children were tested for IgE antibodies to cat, dog, mite, egg white and cow's milk and for IgG antibodies to a panel of common foods.

The aim of the present study was to assess the cross-sectional relation between IgG to a panel of foods and IgE to common allergens (mite, dog, cat, milk and egg) in young children. If there is an association, a procedure might be developed to help identify young children with an increased risk of developing allergic disease in the future.

Subjects and methods

Selection of the study population

The Bokaal study is a prospective birth cohort study, not selected for allergy risk, designed to investigate the effect of short-term cow's milk supplementation of newborn breast-fed infants on the development of cow's milk allergy and atopic manifestations during the first 2 years of life. In the Bokaal study 70 midwives from the western part and centre of The Netherlands (urban and rural villages) asked all pregnant women who intended to breast-feed their child for at least 6 weeks to participate in a double-blind study. All healthy full-term born children were included and randomized; they were either breast-fed and received a standard whey protein dominant cow milk supplementation (containing 11.1 g proteins/100 g powder; Nutrilon Premium®, Nutricia, Zoetermeer, The Netherlands) or were breast-fed and received a placebo (maltodextrin, glucose and mineral solution emulsified with vegetable fats). The cow's milk supplementation or the placebo was given for at least three times during the first 3 days after randomization. Informed consent was obtained from the children's parents before birth. The children were followed up until the age of 2 years. The results of the Bokaal study (presented in another paper [20]) show that early and brief exposure to cow milk proteins in (otherwise) breast-fed children does not increase the risk of atopic disease in the first 2 years of life.

At the age of 1 year a venous blood sample was drawn from all participating children for determination of total IgE and specific IgE for house dust mite, cat, dog, cow's milk

and egg. Also, IgG antibodies to a panel of selected foods were determined in part of the children. 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 [21]. The selected antigens cover the normal spectrum of foods given to infants in The Netherlands.

Children scoring positive (> 1 IU/mL) on at least one of the allergens house dust mite, cat, dog, cow's milk and egg, were IgE-positive and were classified atopic. The atopic group was divided into atopic with eczema and atopic without eczema. All children were seen at the age of 1 year by two of the Bokaal investigators (MdJ and VS), and were scored for the presence of atopic eczema, as defined by Hanifin and Rajka [22]. The nonatopic children in the Bokaal study did not have any family risk of atopy, clinically obvious or possible atopic disease or any positive RAST score ≥ 0.3 IU/mL (for detailed description see [20]).

The original Bokaal study cohort consisted of 1533 randomly selected children born between February 1992 and January 1994. From 1434 children, a venous blood sample was obtained at 1 year of age. Of these 1434 children, 124 were IgE-positive for cow's milk, chicken's egg, cat, dog and/or house dust mite. For the present study these 124 atopic children and a random sample of 150 nonatopic children from the Bokaal study cohort were selected. Due to technical problems or because there was not enough blood available, 10 blood samples (four atopic and six nonatopic) were not used for further analysis. Thus, in the blood samples of 264 children, food-specific IgG antibodies were measured.

Laboratory methods

Total serum IgE was measured as previously described by Stallman and Aalberse [23]. Specific IgE was determined by RAST, as described by Aalberse *et al.* [24], and results were expressed in RAST units per milliliter; one RAST unit represents ≈ 2.4 ng of specific IgE. Therefore a twofold diluted reference serum was incubated with Sepharose-coupled antihuman IgE and ^{125} I-labelled anti-IgE, raised in sheep. Values higher than 1 unit/mL were considered positive. All RAST tests were performed in duplicate.

Food-specific IgG antibodies in sera were measured by enzyme-linked immunosorbent assays (ELISA). Food extracts (10 μ g/mL in PBS) were coated to microtitre plates (Maxisorp, Nunc, Denmark), using 100 μ L per well. After coating, plates were washed three times with washing buffer (PBS/0.02% [v/v] Tween-20). For the determination of specific IgG, 100 μ L of serum sample (diluted 1 : 1000 in PBS/0.05% [v/v] Tween-20) was added to each well and plates were incubated for 1 h under continuous shaking. To remove non-bound serum components, plates were washed three times with washing buffer. For the detection of bound

IgG, wells were incubated with 100 μ L of horse radish peroxidase-labelled antihuman IgG (CLB, Amsterdam, The Netherlands), diluted 1:3500 in PBS/0.02% (v/v) Tween-20/0.2% (w/v) gelatine/6% (w/v) BSA, for 1 h. After washing the plates three times with washing buffer, 100 μ L of 0.01% (w/v) of 3,5,3',5'-tetramethylbenzidine (Merck, Darmstadt, Germany)/0.003% (v/v) of H₂O₂ in 0.11 mol/L sodium acetate pH 5.5 per well was added. The reaction was stopped by adding 100 μ L 2 mol/L H₂SO₄, and optical density was read at 450 nm in an automatic ELISA reader (Bio-Tek Instruments, Winooski VT, USA).

On each plate, apart from the food to be tested, two rows of wells were coated with the wheat protein gliadin (1 μ g/well), and these wells were incubated with a twofold dilution series of a reference serum, containing 100 arbitrary units per millilitre (AE/mL) of IgG to gliadin. This reference dilution curve was used to express all results obtained in our food ELISA in AE/mL. All tests were performed in duplicate.

During our investigations it was found that some sera showed substantial nonspecific IgG binding to the plate material, and therefore all sera were tested in a parallel ELISA procedure in which no food was coated, and the results were corrected for nonspecific binding.

Data analyses

The results of the RAST were dichotomized as IgE-negative or IgE-positive. Children were considered atopic if they were IgE-positive (> 1 IU/mL) for one or more allergens.

The results of the food assays were dichotomized high or low using the 66th centile of the total group as a cut-off value (P66_{total}). The 66th centile was chosen because for all foods, except for meat, the lower boundaries of the 95% confidence interval around the 66th centile [25] were higher than the detection limit of 2.4 AE/mL. Because the 66th centile of IgG to meat was too close to the detection limit, the multivariate analyses were performed without meat in the model.

Some logistic regression analyses were also performed in the group of children without eczema. In these analyses all foods were dichotomized using the 66th centile of this group of children (P66_{ne}).

Logistic regression analysis was used to calculate odds ratios (with 95% confidence intervals) for the univariate and multivariate analyses. Discriminant analysis was used for the probability of being atopic when having high levels of IgG to foods. On the basis of IgG to foods we tried to classify whether a child would be atopic or nonatopic. The discriminant analysis was used to specify the sensitivity and specificity of IgG to foods and the percentage correctly classified.

In all analyses, a *P*-value < 0.05 was regarded as statistically significant. Likelihood ratio statistics were used as a criterion for selection in the logistic regression model.

Data-analysis was performed with SPSS-PC⁺.

Results

General characteristics

The characteristics of the study population are shown in Table 1. Of the 264 children whose blood was available for data analysis, 144 were nonatopic and 120 children were atopic. There was no significant difference in the distribution of boys and girls between the two groups (*P* = 0.4). Cow's milk supplementation had been given to 132 children, 46.5% of the nonatopic and 54.2% of the atopic ones. The geometric mean total IgE for the nonatopic children was 5.6 IU/mL, for the atopic children this was 55.8 IU/mL. Of the 120 atopic children, 56.7% had specific IgE for milk, 54.2% for egg, 19.2% were IgE-positive for cat, 9.2% for dog and 2.5% for mite (data not shown).

Clinically atopic disease was obvious in 39.2% of the atopic children, 64.2% of the atopic children had a family risk of atopy/allergy. Eczema was present in 48.3% of the atopic children, respiratory and gastrointestinal problems in 27.5 and 5.8%, respectively (data not shown).

In Table 2 the characteristics of the IgG food assays are shown. The range in IgG to foods (values of AE/mL) varies per food, e.g. meat ranges from 0.4 to 34.1 AE/mL, while cow's milk ranges from 2.0 to 854 AE/mL. All lower boundaries of the 95% confidence intervals around the 66th centiles of the foods were higher than the detection limit of 2.4 AE/mL, with the exception of meat.

Association between IgG and IgE in all children

In a univariate analysis, all foods were associated with atopy using the 66th centile as a cut-off value (P66_{tot}; Table 3). The highest odds ratios were found for egg white (OR = 7.50) and mixture of wheat and rice (OR = 4.79).

Because all foods were associated with atopy, a multivariate logistic regression analysis was performed with all foods in the model (but not meat) and presence of atopy as the dependent variable (Table 3). Logistic regression analysis showed statistically significant differences in higher IgG levels of egg white (OR = 3.76), mixture of wheat and rice (OR = 2.43) and orange (OR = 2.11). Being atopic was not associated with higher IgG levels of mixture of soybean and peanut, cow's milk and potato.

Inclusion of gender in the regression models did not influence the coefficients for the other variables.

Association between IgG and IgE in children without eczema

The association between IgG to foods and atopy in the group of children without eczema was also analysed. The foods were dichotomized as high or low using the 66th centile for

Table 1. Characteristics of 264 children in the study population (%)

	Nonatopic <i>n</i> = 144 (%)	Atopic <i>n</i> = 120 (%)
Boys	70 (48.6)	64 (53.3)
Girls	74 (51.4)	56 (46.7)
Cow's milk supplementation	67 (46.5)	65 (54.2)
Any RAST*		
0	144 (100)	0 (0)
1	0 (0)	85 (70.8)
2	0 (0)	21 (17.5)
3	0 (0)	13 (10.8)
4	0 (0)	1 (0.8)
Total IgE (IU/mL)**	5.6 (4.3–7.4)	55.8 (43.8–71.1)

*Number of positive RASTs. ** Geometric mean (with 95% CI).

this noneczematous group (P66_{ne}). For all foods the mean was significantly higher in the atopic children without eczema than in the nonatopic children without eczema. Logistic regression analysis showed that higher levels of IgG to foods were still associated with atopy (Table 3). The highest odds ratios were found for orange, egg white and meat (OR = 5.12, OR = 4.04 and OR = 4.79, respectively). Atopy was significantly associated with orange (OR = 3.29), egg white (OR = 2.45) and cow's milk (OR = 2.32), when a multivariate analysis was performed with all foods in the model.

Predictive value of the models

A discriminant analysis was performed to classify on the basis of IgG to foods whether a child was nonatopic or atopic (Table 4). In the model with all foods (cut-off P66_{total}) as independent variables and atopy as the dependent variable,

75.4% of the children could be correctly classified. Sensitivity in this model is 67.5% and specificity is 81.9%.

As to the model with the foods (cut-off P66_{total}) with highest odds ratios (i.e. egg white, mixture of wheat and rice, and orange), 74.2% of the children could be correctly classified. The sensitivity is 65.8% and specificity is 81.3%.

In the model with all noneczematous children (cut-off P66_{ne}), 72.3% of the children could be correctly classified. The sensitivity and specificity were, respectively, 67.7 and 74.3%.

Discussion

In this study we have investigated the association 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) and specific IgE to milk, egg, cat, dog and/or mite in 1-year-old children. We found a strong relationship between IgG to foods and atopy, for all foods, especially for

Table 2. Characteristics of IgG food assays (AE/mL), with different cut-off points

	Minimum ^a	P66 _{ne} ^b	P66 _{total} ^c	P75 ^a	P90 ^a	Maximum ^a
Mixture of wheat & rice	0.6	7.8	10.6	13.9	37.5	109.9
Mixture of soy bean & peanut	0.8	6.5	8.8	12.1	39.2	254.0
Cow's milk	2.0	58.6	62.0	74.6	271.6	854.0
Egg white	0.3	3.8	5.9	7.9	23.0	51.6
Meat	0.4	1.8	1.8	2.2	4.9	34.1
Orange	0.5	4.5	5.0	6.0	17.0	34.2
Potato	0.4	3.3	3.6	4.5	15.3	28.6

^aMinimum, 75th centile, 90th centile and maximum of the total group (*n* = 264). ^b66th centile of the group of children without eczema (*n* = 206). ^c 66th centile of the total group (*n* = 264).

Table 3. IgE (atopy) in relation to IgG to foods; odds ratios (with 95% CI) ($n = 264$)

	All children ($n = 264$)		Children without eczema ($n = 206$)	
	Atopy ^a	Atopy ^b	Atopy ^c	Atopy ^d
Mixture of wheat & rice	4.79 (2.76–8.31)*	2.43 (1.28–4.61)*	3.51 (1.88–6.56)*	1.62 (0.77–3.38)
Mixture of soybean & peanut	3.27 (1.92–5.57)*	1.53 (0.79–2.96)	1.93 (1.05–3.58)*	1.13 (0.54–2.36)
Cow's milk	2.13 (1.27–3.58)*	1.57 (0.84–2.93)	2.13 (1.15–3.95)*	2.32 (1.12–4.82)*
Egg white	7.50 (4.20–13.41)*	3.76 (1.88–7.49)*	4.04 (2.15–7.58)*	2.45 (1.16–5.14)*
Meat	3.82 (2.26–6.47)*	–	4.79 (2.53–9.05)*	–
Orange	3.97 (2.31–6.82)*	2.11 (1.10–4.06)*	5.12 (2.70–9.71)*	3.29 (1.59–6.82)*
Potato	4.14 (2.40–7.15)*	1.75 (0.90–3.42)	3.89 (2.08–7.29)*	1.93 (0.92–4.07)

*Significant at $P < 0.05$. ^aFoods dichotomized positive or negative using the 66th centile (of the total group) as a cut-off value, univariate analysis. ^bMultivariate analysis, all foods included in the model, 66th centile of the total group. ^cFoods dichotomized positive or negative using the 66th centile (of the group of children without eczema) as a cut-off value, univariate analysis. ^dMultivariate analysis, all foods included in the model, 66th centile of the group of children without eczema.

egg white and mixture of wheat and rice. Furthermore we tried to classify on the basis of IgG to foods, whether a child was atopic or nonatopic. Atopy could be correctly classified for children with high levels of IgG to foods in 75.4% of the 1-year-old children.

Not all foods are equally important in classifying atopy in the children. Egg white, mixture of wheat and rice, and orange are selected in a stepwise logistic regression analysis. These three foods classify 74.2% of the children correctly, whereas with a model with all foods 75.4% of the children are correctly classified.

All foods selected for logistic regression analysis were dichotomized as high or low using the 66th centile as a cut-off value. A lower cut-off, e.g. the median rather than the 66th centile, results in most cases, in 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 75th centile, were less discriminating than the 66th centile as a cut-off value, which led to a decreased sensitivity and specificity.

A limitation in our study was that most of the 120 atopic children were IgE-positive for egg and milk. We measured both IgG and IgE to cow's milk and to egg white. However, it has been well established that IgE to egg and milk are closely associated with the development of IgE to inhalant allergens [3,26–28]. In our study all but eight children in the atopic group had IgE to egg and/or milk, this group of atopic children, however, being too small a group for additional analysis. It is therefore important to note that not only IgG antibodies to egg and milk are associated with atopic manifestations, but that also IgG antibodies to other foods (orange, mixture of wheat and rice in the present study, legumes and mixture of wheat and rice in

	Sensitivity	Specificity	% correctly classified
<i>Total group^b</i>			
All foods ^a	67.5	81.9	75.4
Egg, orange, mixture of wheat & rice	65.8	81.3	74.2
<i>Noneczematous^c</i>			
All foods ^a	67.7	74.3	72.3

^aAll foods included in the discriminant analysis (meat excluded). ^bFoods dichotomized positive or negative using the 66th centile of the total group as a cut-off value ($n = 264$).

^cFoods dichotomized positive or negative using the 66th centile of the noneczematous group as a cut-off value ($n = 206$).

Table 4. Discriminant analysis: IgE for cat, dog, house dust mite, milk and/or egg and IgG to foods

the study of Calkhoven *et al.* [19]) are equally good predictors.

The children were studied at 1 year of age. At this age IgE antibodies to inhalant allergens are rare [29] and both the concentration and prevalence of specific antibodies increase from the second to the fifth year [29,30]. In this study only 32 of the 1434 children of the original Bokaal study cohort had IgE antibodies to cat, dog and/or mite (and/or egg and milk).

Several studies [19,29–34] have reported an association between IgG to certain allergens and IgE to inhalants, but in these studies the children were at a high risk for the development of specific IgE (either they had a parental history of atopic disease [19,29,31] and/or they had eczema (or asthma) themselves [19,32–34]). In the present study both atopic children with and without eczema were studied. A logistic regression analysis was performed without the eczematous atopic children. The results indicated that atopic children without eczema have higher levels of IgG to foods than nonatopic children, although the specificity and percentage of children correctly classified were lower in this model than in the model with all children (the eczematous children included). We also performed an analysis without the children with persistent eczema (persistent meaning the eczema was still present at the age of 2 years). If these children were not taken into account, the association between high levels of IgG and atopy still existed.

Why IgG to foods is associated with IgE to inhalant allergens is not clear. One possibility is that both reflect hyperactivity of the mucosal immune system or an increased permeability to macromolecules (as suggested by Salvaggio *et al.* [35]).

In all children IgG antibodies to foods could be measured, so if IgG antibodies develop before or at the same time as IgE antibodies, as is suggested in other studies [29,33], high levels of IgG to foods might be used as a predictor of IgE-mediated allergy in initially nonatopic children. The results in this study are promising in this respect and indicate that there is a relation between increased IgG antibody level to foods and IgE antibodies to inhalant allergens, egg and milk, even in children without eczema. Since only a follow-up study can show whether increased levels of IgG antifoeds predict the future development of IgE to inhalant allergens, such a study is currently being carried out at our department.

Note added in proof

Since the acceptance of this manuscript, a crystal structure for the alpha-chain of FcεRI has been published [36], although the co-ordinates or stereo representations of this structure are not yet available. It is impossible to comment on how the structure differs from model predictions and if the conformation of the predicted binding regions for

IgE vary significantly from those projected in the current study.

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