

IgG Antibodies Against Food Antigens are Correlated with Inflammation and Intima Media Thickness in Obese Juveniles

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QUESTIONS:

Association between IgG-antibodies to foodstuffs, low-grade inflammations and early atherosclerotic lesions in children with and without overweight.

RESULT:

Overweight children have a significantly increased IgG-antibody level to foodstuffs compared with children of normal weight. In the group of overweight children, the intima media thickness (IMT) of the carotid artery and the CRP level were significantly increased as well. IgG-titres correlate with CRP and IMT. IgG-antibodies to foodstuffs are closely associated with low-grade inflammations and IMT. These findings lead to the conclusion that IgG-antibodies to foodstuffs have an influence on the development of overweight and atherosclerosis.

RELEVANCE FOR IMUPRO 300:

Overweight persons have more and higher IgG-titres compared to persons of normal weight. Furthermore, the IgG-titres correlate with the CRP levels that indicate an inflammatory process in the body. This corroborates the thesis that the consumption of foodstuffs to which IgG-antibodies are present triggers an inflammatory process. This results in the development of insulin resistance causing affected persons to become more and more obese and / or having difficulties in losing weight.

A change in diet based on ImuPro 300 stops this inflammatory process; the patients succeed in losing weight.

The study results also indicate an association of IgG-antibodies and atherosclerosis. IgG-titres correlate with CRP levels and IMT. Increased values may indicate the onset of atherosclerosis.

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Key words

- coronary artery
- cardiovascular incidences
- oxidative

Abstract



Objective: Systemic low grade inflammation may contribute to the development of obesity, insulin resistance, diabetes mellitus and atherosclerotic vascular disease. Food intolerance reflected by immunoglobulin G (IgG) antibodies may predispose to low grade inflammation and atherogenesis. We examined the relationship between IgG antibodies specific for food components, low grade inflammation and early atherosclerotic lesions in obese and normal weight juveniles.

Research Methods and Procedures: We determined IgG antibodies directed against food antigens, C-reactive protein (CRP) and the thickness of the intima media layer (IMT) of the carotid arteries in 30 obese children and in 30 normal weight children.

Results: Obese juveniles showed a highly significant increase in IMT ($p=0.0001$), elevated CRP values ($p=0.0001$) and anti-food IgG antibody concentrations ($p=0.0001$) compared to normal weight juveniles. Anti-food IgG showed tight correlations with CRP ($p=0.001/r=0.546$) and IMT ($p=0.0001/r=0.513$) and sustained highly significant in a multiple regression model.

Discussion: We show here, that obese children have significantly higher IgG antibody values directed against food antigens than normal weight children. Anti-food IgG antibodies are tightly associated with low grade systemic inflammation and with the IMT of the common carotid arteries. These findings raise the possibility, that anti-food IgG is pathogenetically involved in the development of obesity and atherosclerosis.

received 28.08.2007
 first decision 11.10.2007
 accepted 26.10.2007

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DOI 10.1055/s-2007-993165
 Published online: 2007
 Exp Clin Endocrinol Diabetes
 © J. A. Barth Verlag in
 Georg Thieme Verlag KG
 Stuttgart · New York
 ISSN 0947-7349

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Introduction



Low grade inflammation may play a causal role in the development of obesity, insulin resistance, diabetes mellitus and atherosclerosis [1–3]. In obese subjects, adults as well as children, inflammatory markers, like C-reactive protein (CRP) correlate with the degree of obesity and insulin resistance and normalise after weight reduction [4–9]. We previously observed a close correlation between the intima media thickness (IMT) of the common carotid arteries and CRP, indicative for a pre-atherosclerotic status in obese children [10]. Our findings are confirmed, as these childhood obesity related effects have been shown to contribute to the development of atherosclerosis [11–14]. Despite the overwhelming evidence that low grade inflammation is associated with diabetes mellitus and atherosclerosis, factors and mechanisms which initiate and uphold low grade systemic inflammation are still under discussion.

Recently, immunoglobulin G (IgG) antibodies against food antigens have been suggested to cause low grade inflammation in the irritable bowel syndrome by subtle mucosal inflammation [15]. Food elimination therapy based on IgG testing was able to improve the symptoms of the irritable bowel syndrome [16]. IgG-mediated food intolerance may be explained by low level absorption of food macromolecules from the gut [17]. Thus, IgG antibodies to some food components are detectable in healthy individuals although at lower levels, the role of this class of antibodies remains highly controversial [18–20]. Aim of the present study was to examine, whether IgG mediated food intolerance is associated with inflammation and pre-atherosclerosis in obese juveniles. We determined specific IgG antibodies against food antigens as well as plasma CRP levels and IMT of the carotid arteries in obese and normal weight children.

Table 1 Clinical and biochemical characteristics, anti-food IgG and IMT in normal weight and obese children

	Normal weight (n = 30)	Obese (n = 30)	P
age (years)	14.4 ± 2.6	12.8 ± 2.9	0.024 ⁺
body mass index (kg/m ²)	20.5 ± 1.7	30.1 ± 4.6	<0.001*
BMI-SDS	0.71 ± 1.02	5.75 ± 1.55	<0.001*
systolic blood pressure (mmHg)	125 ± 8	128 ± 16	n.s.
diastolic blood pressure (mmHg)	67 ± 7	68 ± 14	n.s.
intima media thickness (mm)	0.49 ± 0.08	0.61 ± 0.09	<0.001 ⁺
triglycerides (mg/l)	0.84 ± 0.38	1.20 ± 0.58	0.014 ⁺
cholesterol (mg/l)	1.62 ± 0.27	1.66 ± 0.27	n.s.
LDL Cholesterol (mg/l)	1.00 ± 0.23	1.02 ± 0.19	n.s.
HDL Cholesterol (mg/l)	0.46 ± 0.09	0.43 ± 0.11	n.s.
plasma glucose (g/l)	0.82 ± 0.24	0.89 ± 0.10	n.s.
insulin (mU/l)	13.1 ± 10.7	30.2 ± 27.2	0.033 ⁺
CRP (mg/l)	1.2 ± 1.7	3.6 ± 3.0	<0.001*
anti-food IgG (mg/l)	600 ± 327	1451 ± 972	<0.001*

Results are expressed as mean ± SD

⁺two tailed Student's t-test for independent samples

*Mann-Whitney-U test; n.s. not significant

Research Methods and Procedures

Patients

We investigated 30 obese juveniles and 30 normal weight children at the Clinical Institute of Medical and Chemical Laboratory Diagnostics and the Department of Pediatrics, Medical University of Graz. Obesity was defined as a body mass index (BMI) value greater than the 97th percentile. BMI and BMI-standard deviation score (BMI-SDS) were calculated by the Growth Analyser Program. BMI-SDS represents an age and sex specific standard deviation. Obese subjects attended the clinics to get dietary advice. The normal weight control persons came for minor surgical interventions and were otherwise healthy. All patients included in the study had to be free of any infectious diseases at least for three weeks prior to blood sampling. The study was approved by the ethics committee of the University of Graz (serial number of approval: 13–200 ex 02/03). Blood collection was performed after written informed consent was given by the patients.

Blood collection

Blood was obtained by venous puncture, immediately centrifuged at 3500 × min⁻¹ at ambient temperature and the serum was stored at -25 °C until analysis.

Laboratory procedures

Glucose (hexokinase method), cholesterol, and triglycerides were measured enzymatically (Roche Diagnostics, Mannheim, Germany). LDL cholesterol and HDL cholesterol were measured by a combined ultracentrifugation and precipitation method [21]. CRP was measured with a particle-enhanced immunoturbidimetric assay (Tinaquant[®], C-reactive protein ultra sensitive assay, Roche Diagnostics, Mannheim, Germany). Insulin was determined by radioimmunoassay (INSI-CTK IRMA; Sorin Diagnostics Düsseldorf). Serum IgG₁₋₄ antibodies against 277 food antigens were detected using a commercial available enzyme immunoassay (Imupro 300, Evomed/R-Biopharm, Darmstadt, Germany).

Carotid artery ultrasound

The bulbous near common carotid arteries (CCA) on both sides were scanned with a 12–5-MHz broad-band linear transducer on a HDI 5000 (ATL, Bothell, Washington, DC, USA). Longitudinal images directed through the centre of the artery were taken at each vessel site. Measurements were made from stored digital images by an experienced reader. The intima media thickness (IMT) was assessed at the far wall as the distance between the interface of the lumen and intima, and the interface between the media and adventitia. The maximal IMT was recorded at each of the vessel segments and averaged for the left and right carotid artery. The lumen diameter was calculated as the inter-adventitial diameter minus twice the maximum far wall IMT. All diameters were obtained during the diastole to avoid image blurring due to systolic arterial wall motion, and to minimize the influence of blood pressure.

Statistical analysis

Data are presented as means ± standard deviations. Continuous variables were compared using Students t-test for independent samples or Mann-Whitney-U Test depending on the contribution of data. Correlations between variables were determined by linear regression analysis according to Pearson and subsequent multiple regression analysis. P-values less than 0.05 were considered statistically significant. Analyses were performed using SPSS for Windows.

Results

Clinical and biochemical characteristics of obese and normal weight children are given in **Table 1**. Compared to normal weight children, obese children showed significantly increased triglycerides (p=0.014) and insulin (p=0.033). Blood pressure, cholesterol and plasma glucose were also increased, but did not reach statistical significance. Obese juveniles showed a highly significant increase in IMT (p<0.001), elevated CRP values (p<0.001) and anti-food IgG antibody concentrations (p<0.001) (● **Fig. 1**). CRP plasma concentrations were 3-fold higher in obese (3.6 ± 3.0 mg/l) than in normal weight children (1.2 ± 1.7 mg/l). Anti-food IgG concentrations were found about 2.5-fold higher

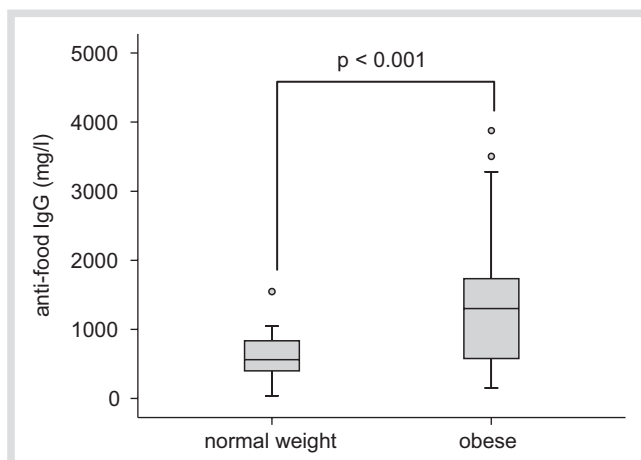


Fig. 1 Box and whiskers plot of serum anti-food IgG values in normal weight controls and obese juveniles. p two-tailed Student's t -test for unpaired samples.

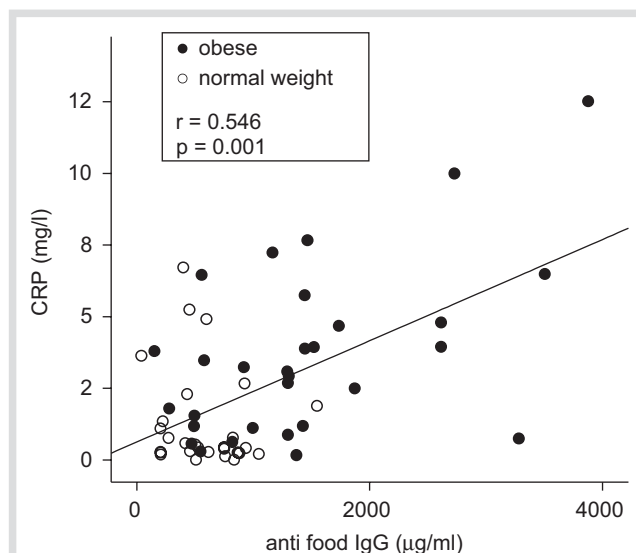


Fig. 2 Correlation between IgG antibodies against food antigens and CRP in normal weight (○) and obese (●) juveniles. r Pearson correlation coefficient.

Table 2 Regression analysis of anti-food IgG antibodies in obese patients and normal weight controls

	r	p
BMI (kg/m ²)	0.304	0.018
BMI SDS	0.400	0.002
systolic blood pressure (mmHg)	0.569	0.034
diastolic blood pressure (mmHg)	0.163	0.579
intima media thickness (mm)	0.513	<0.001
triglycerides (mg/l)	0.030	0.844
cholesterol (mg/l)	0.062	0.683
LDL-Cholesterol (mg/l)	0.118	0.475
HDL-Cholesterol (mg/l)	0.109	0.510
plasma glucose (g/l)	0.036	0.824
insulin (mU/l)	0.033	0.867
CRP (mg/l)	0.546	0.001

r Pearson correlation coefficient; p univariate ANOVA

in obese ($1451 \pm 927 \mu\text{g/ml}$) than in normal weight children ($600 \pm 327 \mu\text{g/ml}$). Anti-food IgG were not affected by gender (obese group $p=0.514$; normal weight group $p=0.605$). Thus obese and normal weight children slightly but statistically significant differ in age we determined possible age-effects on IgG concentrations. Correlation analyses by linear regression revealed no age-effect on anti-food IgG in obese ($p=0.303/r=0.194$) and normal weight ($p=0.763/r=0.057$) juveniles. Correlation analyses between anti-food IgG and various variables are shown in **Table 2**. No correlation is seen between anti-food IgG and plasma glucose, insulin, triglycerides, cholesterol, LDL-cholesterol and HDL-cholesterol. Anti-food IgG showed positive correlations with BMI-SDS ($p=0.002/r=0.400$), CRP ($p=0.001/r=0.546$) (● **Fig. 2**) and IMT ($p<0.001/r=0.513$) (● **Fig. 3**). Interestingly, anti-food IgG were correlated with systolic blood pressure ($p=0.034/r=0.569$), but not with diastolic blood pressure ($p=0.579/r=0.163$). Variables found to correlate with anti-food IgG were included in a multiple regression model. Multiple testing revealed that the correlation with systolic blood pressure and BMI-SDS was not robust. A highly significant correlation by multiple testing was found between CRP ($p<0.001$), IMT ($p=0.001$) and anti-food IgG ($p=0.022$) (**Table 3**).

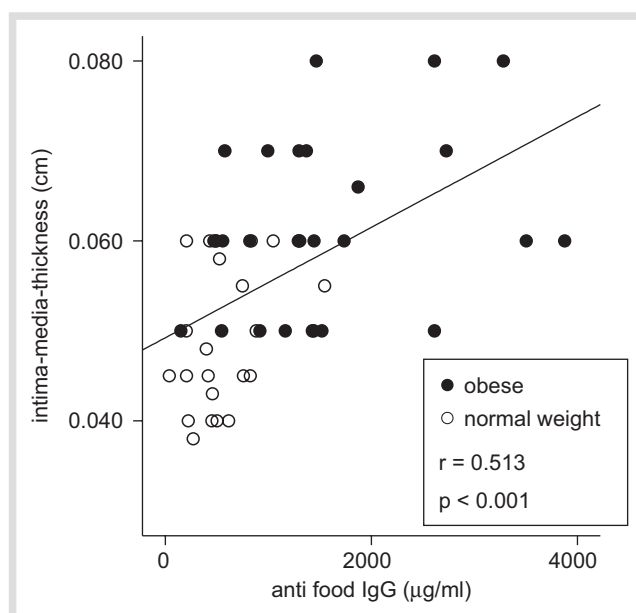


Fig. 3 Correlation between IgG antibodies against food antigens and the IMT of the common carotid arteries in normal weight (○) and obese (●) juveniles. r Pearson correlation coefficient.

Discussion

In the present study we show that obese children have significantly higher IgG antibody values directed against food antigens than normal weight children. Anti-food IgG antibodies were found to be tightly associated with low grade systemic inflammation and with the IMT of the common carotid arteries in obese and normal weight juveniles. Immunological reactions against food components are discussed to contribute to the pathophysiology of the irritable bowel syndrome (IBS) [17]. Atkinson et al. show that a food elimination therapy based on the presence of IgG antibodies to particular food components was effective in reducing IBS symptoms [16]. IgG-mediated food

Table 3 Multiple stepwise linear regression analysis to evaluate correlations with anti-food IgG in obese patients and normal weight controls

	beta Coefficient	P
constant (anti-food IgG)		0.022
intima media thickness (mm)	0.409	0.001
CRP (mg/l)	0.459	<0.001

intolerance may be explained by low level absorption of food macromolecules from the gut causing low grade chronic inflammation [17]. A number of dietary components seem to be able to modulate the inflammatory response in humans, thereby affecting cardiovascular risk [22]. We hypothesize that the gut might represent one of the key organs to induce and to perpetuate low grade chronic inflammation. The mechanisms which induce and maintain food tolerance during lifetime are not well understood and the meaning of IgG against food is in discussion [23–25]. Recent findings suggest that low grade systemic inflammation represents an essential cause rather than a consequence of various pathophysiologies like type 2 diabetes and atherosclerosis [1, 3, 7].

Acute phase reactants have been shown to predict future weight gain and the development of type 2 diabetes [5, 7, 26]. We show that CRP as an acute phase reactant was highly significant increased in obese juveniles and tightly correlated with anti-food IgG. It has been consistently reported that obesity, in adults as well as in children, is associated with increased concentrations of CRP [4, 27]. CRP is an independent indicator for future vascular events and a systemic marker reflecting cytokine mediated processes [14]. Obesity related increased CRP concentrations have been discussed as a consequence of increased hepatic synthesis in response to interleukin-6 release from adipose tissue [28]. CRP may also actively be involved in the development of atherosclerosis and its clinical complications as it is found in the vessel wall even at early stages of plaque formation [29]. Further, CRP contributes to atherogenesis as it is chemotactic for monocytes, induces complement activation, promotes foam cell formation and induces the expression of various adhesion molecules [30–32]. Hence, it is not surprising that we recently found a close correlation between CRP and the IMT of the common carotid arteries, a well established non-invasive marker for the beginning, progression and burden of atherosclerotic vascular changes [10, 33, 34]. Beyond, the increased IMT in obese juveniles we show here that anti-food IgG are highly significant and tightly correlated with IMT in obese and normal weight juveniles.

Taken together, our results suggest that particular food intolerance increases anti-food IgG in obese juveniles. These anti-food IgG are linked to low grade inflammation and atherogenesis. These findings raise the possibility, that anti-food IgG are pathogenetically involved in the development of obesity and underline the notion that atherosclerosis can start much earlier in life than hitherto assumed. We are well aware of the fact that the impact of anti-food IgG in the pathophysiology of atherosclerosis is not clear to date. Especially the impact of anti-food IgG in regard to metabolic changes which contribute to type 2 diabetes and atherogenesis, like elevated serum lipid levels and insulin resistance remains elusive. As described above, our study addresses a relationship between anti-food IgG, obesity, systemic inflammation and early atherosclerosis. Therefore our results need to be reproduced in larger cohorts, including adults with more advanced stages of atherosclerosis. However, once

confirmed, our findings might have important implications in the clinical management of weight reduction and prevention of atherosclerosis. Especially, as described for the IBS above, a dietary elimination therapy based on the presence of IgG antibodies to food components may be indicated. Such a dietary therapy may be effective in reducing low grade inflammation and thereby preventing clinical consequences like type 2 diabetes and atherogenesis.

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