
# Cambridge Nutritional Sciences Evidence-Based Clinical Relevance of Food Specific Serum IgG Antibodies

**May 2017**

# Contents

## Executive Summary

- FoodPrint™ and Food Detective™ products measure the physiological state of IgG antibodies to food in the body. They do not and should never be used to identify classical IgE mediated food allergies.

- There is a substantial body of published literature to indicate that the measurement of food specific IgG antibodies are a safe and clinical useful tool for the assessment of a wide variety of common conditions.

- Elimination diets based on the results of food specific IgG antibody measurements have demonstrated effectiveness in alleviating a wide variety of common conditions.

- The overwhelming majority of the negative published literature surrounding food IgG antibody measurements refers specifically to the use of IgG4 in such assessments or to the use of IgG in the assessment of food allergy. This literature therefore, should not form part of a review for the utility of food specific IgG for the investigation of food intolerance.

- **On the basis of the relevant information available, the intended use of these devices is supported by the literature.**

Dr Nigel Abraham

Scientific Director

Cambridge Nutritional Sciences

## Summary of Background and Definitions

A food allergy is an adverse immune response to a food component (allergen), which can cause a variety of allergic responses. There are two common antibodies associated with adverse immune reactions to food, IgE and IgG.

IgE-mediated food reactions, type I hypersensitivity, are immediate after exposure to the allergen and the symptoms displayed are classically associated with an allergic reaction such as anaphylaxis.

Tests for specific IgE antibodies are used to show an abnormal physiologic response to an allergen.

IgG-mediated food reactions, type III hypersensitivity, usually occur several hours or days after exposure and can be caused by multiple allergens. Type III hypersensitivity is associated with an inflammatory response which has been shown to be related to conditions such as bloating, diarrhoea, Irritable Bowel Syndrome (IBS) and headaches/migraines.

Tests for specific IgG antibodies are used to show an abnormal physiologic response to an antigen.

Food intolerance is a general term in the public domain which is used to describe type III hypersensitivity as well as reactions to antigens which are non-immune mediated.

## Product Information and Intended Use

### Point of Care Testing

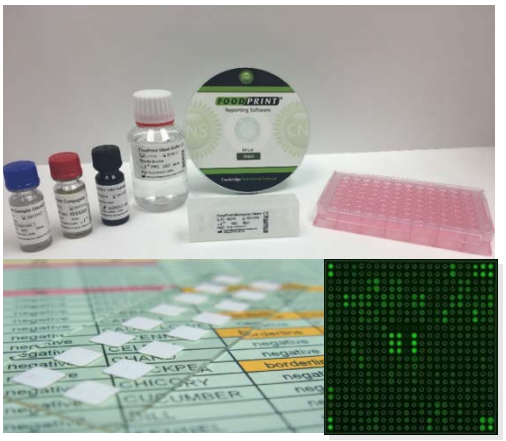
The Food Detective product utilises proven ELISA technology to identify raised food-specific IgG antibodies that are produced in response to allergens. Evidence shows that these antibodies are involved in various conditions such as bloating, diarrhoea, IBS and headaches/migraines.


The test is not a classic allergy test (type 1 hypersensitivity) and is for individuals over the age of two.

There are a variety of CE marked IgG products and tests currently available on the market.

### Food Intolerance Microarray

FoodPrint™ is a laboratory-based system developed and manufactured by Cambridge Nutritional Sciences, which utilises an innovative, colorimetric microarray-based ELISA technology for the measurement of food-specific IgG antibodies in human serum, plasma or whole blood from a finger prick sample.




The flexibility of the system permits a wide range of food panels to be offered, with over 220 foods, together with vegan, vegetarian and herbs/spices options.

## Background and Definitions

An adverse food reaction is a general term describing clinically abnormal responses to an ingested food that may be related to allergic food hypersensitivity (food allergy) or non-allergenic food hypersensitivity (food intolerance). Food allergy is an immunologic reaction that involves the immunoglobulin E (IgE) mechanism, of which anaphylaxis is the classic example. Such reactions are classified as a type I hypersensitivity as defined by Gell and Coombs in (1963).

Measurement of food-specific IgE antibodies by in vitro assays or skin testing are the routine procedures used to diagnose food allergy. These diagnostic tests, indicate the presence of food-specific IgE antibodies, but they do not establish the diagnosis of food allergy. The final proof of the clinical relevance of the reported history and the detected food-specific IgE is provided by a positive controlled food challenge, as stated by Bindslev-Jensen et al. (2004).

IgG mediated reactions are a distinct entity, with a very different clinical picture, they are usually defined as 'delayed reactions' with a less severe symptom outcome compared to some IgE mediated reactions. Symptoms can typically affect many different body systems and are regarded as an example of a type III hypersensitivity reaction. These types of reactions are characterised by the production of immune complexes with food specific IgG antibodies activating the complement pathway and thus initiating low grade inflammatory reactions. Paganelli et al (1981), demonstrated that ingested food molecules are at the core of such circulating immune complexes and pointed out that whilst;

*"The formation of an antigen-antibody complex in the circulation is a normal physiological method of antigen elimination, there is a great deal of evidence suggesting that increased levels of circulating immune complexes are associated with a variety of diseases in which the complexes, once deposited in the tissues, cause damage by activating complement and other effector mechanisms."*

Food intolerance, is often used to describe a type III hypersensitivity when in reality this term refers to an abnormal physiologic response to an ingested food or food additive. Unlike IgG mediated reactions such reactions are considered to not demonstrably involve the immune system, an example being lactose intolerance where there is a deficiency in the enzyme responsible for breaking down ingested lactose. The term food intolerance however, is useful to help distinguish IgG mediated reactions from classical allergies. Food sensitivity or hypersensitivity is a term which is now increasingly used to distinguish IgG reactions from both IgE reactions and non-immune mediated reactions.

## Immunoglobulin G and Subclasses

IgG antibodies comprise 70–75% of the immunoglobulins in the serum and are the fundamental antibodies of secondary immune response. Four subclasses of immunoglobulin G are distinguished: the IgG1, IgG2, IgG3, and IgG4 subclasses amount to ~66%, ~23%, ~7%, and ~4% of the IgG antibody pool, respectively. IgG1 and IgG3 have strong pro-inflammatory properties activating the complement pathway. IgG4 on the other hand is a unique molecule that has protective, anti-inflammatory properties and most importantly does not activate complement and is involved in the generation of tolerance for IgE mediated reactions. This distinction is vitally important when discussing the validity of food specific IgG antibody assessments.

**Reviewers frequently fail to understand this important difference often citing references specific to IgG4 as evidence against IgG testing in general. It must be stressed that when assessing the validity**

**of assays for total food specific antibodies (IgG 1-4), such as the Food Detective product, that these statements or published works relating to IgG4 specifically have no relevance and are not applicable in upholding an argument against the use of IgG testing.**

In addition, position statements and reviews of allergy that are referring to IgE mediated reactions should not be confused with IgG reactions, as total IgG (subtypes 1-4) assessments do not have any diagnostic relevance to the investigation of this type of allergy (IgE). This point is frequently confused and misunderstood in publications and online reviews.

## Contact Between Food Antigens and the Immune System

In normal conditions, consumed proteins, including potential food antigens, are completely degraded in the digestive tract to oligopeptide fragments. Due to intestinal proteolytic enzyme activity, the latter are broken to di- and tripeptides and amino acids, and then absorbed by enterocytes. Enterocytes are cells which line the intestine and form an internal barrier. Further degradation takes place in enterocytes, to amino acids and dipeptides which enter portal circulation and then are carried to the liver. However about 15% of consumed protein is incompletely digested, including a proportion of food antigens. This is important as a certain amount of food antigens, which were not destroyed by digestion with enzymes, bile salts, and low gastric pH, penetrate the epithelium of the digestive tract and reach the body's internal environment. (Brzozowski et al 2011)

There are three pathways for food antigens to penetrate the digestive tract epithelium.

1. Capture of antigens by Peyer's patch M cells.
2. Capture of antigens from the digestive tract by the dendritic cell processes localized between enterocytes.
3. Capture of antigens by enterocytes.

When enterocytes are damaged and the connections between them weakened, for instance due to inflammatory processes, food antigens may fall between the cells. When this occurs partially digested food antigens can penetrate the digestive tract. Here they encounter the cells of the immune system known as the gut-associated lymphoid tissue (GALT).

The role of GALT is to maintain the immune homeostasis between defending the organism from pathogens which have penetrated the digestive tract, and inducing and maintaining the immune tolerance for innocuous antigens. Therefore, food antigens will be treated by GALT either as innocuous antigens and induce tolerance, or as pathogens. When treated as pathogens an immune response is triggered this may be either a normal defence reaction or an excessive defensive reaction, the latter of which gives rise to type III hypersensitivity. (Agarwal S & Mayer L, 2008).

## The Role of IgG in Triggering Defence Reactions to Food Antigens

IgG antibodies are the main line of acquired defence and a body's specific humoral response to pathogens. As described above in normal conditions the digestive tract epithelium is impermeable to antigens, whereas when it is damaged antigens can permeate under the epithelium and come into contact with immune system cells. This leads to activation of the immune system and production of specific defensive IgG antibodies (sIgG). The subsequent contact of these antibodies with the antigen causes reactions involving the creation of antigen-antibody immune complexes, activation of the

complement protein cascade and effector cells, such as neutrophils, lymphocytes, macrophages, as well as eosinophils and platelets. As a result, the immune complexes are phagocytosed and then destroyed in the reticuloendothelial system. Simultaneously, the inflammatory process caused by the immune reaction between sIgG and food antigens can facilitate further damage and increased permeability of the digestive tract mucosa to food antigens, further exacerbating the response.

Therefore, the presence of specific IgG antibodies directed against food antigens reflects a defence reaction to antigens penetrating due to the damage of the epithelial barrier. The IgG response to food antigens reflects the damage to the mucosa and develops secondary to it. It is also associated with the normal removal from the body of food antigens, which have accidentally penetrated the barrier of the mucosa, while the selectivity of response to certain food antigens may come from the type and quantity of a penetrating antigen and its resistance to digestion.

This concept is well supported by the results obtained by Zuo et al. (2007) who investigated the concentrations of sIgG against 14 food antigens in patients with IBS and functional dyspepsia, compared to a group of healthy patients. IBS and dyspepsia are both conditions where the digestive system is compromised. In all patients from both studied groups as well as in controls, the presence of sIgG antibodies directed against food antigens was confirmed. Importantly, statistically significantly higher levels were observed in patients with IBS and dyspepsia than in controls. The authors did stress the selectivity of response to only some food antigens, may be associated with dietary habits or other factors. The study was not aimed to see if there was a link between symptoms, but it did not reveal any correlation between the severity of the symptoms of functional dyspepsia, irritable bowel syndrome and sIgG levels. Total IgE concentrations in controls and studied groups did not differ statistically and were within normal ranges.

## The Role of IgG Antibodies in Food Allergy

Arguments against IgG often involve IgE reactions. It is therefore important to understand in more detail their similarities, but also the key areas in which these reactions differ.

To be classified as a food allergy, two conditions must be met:

1) involve the immune system

2) be an abnormal reaction

A food allergy meets these criteria as it is an immunologically conditioned, abnormal reaction to food allergens. Reactions mediated by specific IgG antibodies meet the first condition of a food allergy as they are directed against food antigens and are therefore immune reactions by nature. However, as they are normal reactions associated with the exposure to food antigens, they do not meet the second condition of the food allergy definition.

This is relevant as the great majority of published papers where it is concluded that food IgG antibodies have no validity or value are referring specifically to the diagnosis of food allergy.

**It therefore follows that the measurement of food specific IgG antibodies has no role to play nor should it be intended for the diagnosis of classical allergy.**

Below are some typical examples of papers often cited which use IgG in this incorrect manner.

In a 2016 research article by Czaja-Bulsa et al, the authors hypothesised that Food IgG and IgG4 antibodies may be related to the presence of gastrointestinal inflammatory diseases. The objectives were: 1. An analysis of wheat and rice IgG and IgG4 in healthy children, children with IgE-mediated wheat allergy (WA), coeliac disease (CD) and Helicobacter pylori infection (HP). 2. Usability of wheat IgG and IgG4 in wheat allergy (WA) diagnostics.

**The paper concluded:**

> *"The evidence therefore strongly points to the fact that food IgG measurements do not have any clinical significance for the diagnosis of food allergy. Wheat IgG and IgG4 are useless for wheat allergy diagnostics."*

However, since food IgG measurements are not intended for the diagnosis of food allergy and indeed should not be used for such, this is not relevant to the assessment of validity for such IgG measurements.

What is also of interest in this paper and others is that despite clearly showing that elevated food IgG antibodies are seen in gastrointestinal conditions such as Coeliac Disease and Helicobacter pylori infections and indicating that such elevated levels are associated with gastrointestinal inflammation, no mention of this was made in the conclusion. One paper that demonstrates the correlation between elevated food IgG levels and gut inflammation is that of Wilders-Trusching et al. (2008) looking at obese children.

**The paper concluded:**

> *"Evidence clearly demonstrates the potential clinical utility and therapeutic role for the measurement of food IgG antibodies in guiding dietary elimination for investigation of a wide variety of gastrointestinal conditions related to inflammation, characterised by elevated food specific IgG antibodies."*

Antico et al. (2011) examined 73 patients reporting with skin symptoms who associated them with the consumption of foods. The reported symptoms were rash, itching of the skin, and erythema. All patients were subjected to skin tests for food and inhalation allergens, titrations of sIgE and sIgG4, open oral provocation challenges with foods for which sIgG were detected, and double-blind placebo controlled food challenges (DBPCFC) with foods for which the open food challenges were positive.

**The paper concluded:**

> ***"****Titrating sIgG4 in adult patients is not useful clinically in the diagnosis of food allergy or intolerance. The titration of sIgG4 should not be a part of the diagnosis and therapy of adult patients with allergy-related skin disorders."*

The paper fails to highlight that high sIgG4 values are associated with asymptomatic sensitisation and effective immunotherapy, which is indicative rather of a protective or blocking role of these antibodies. Children with a high sIgG4 to sIgE ratio tolerate the sensitizing foods better. High sIgG4 in children with IgE-mediated allergy is a predictive factor of a future tolerance.

**These statements and the work of Antico et al (2011) emphasise the unique function and role of IgG4. It is vitally important therefore when reviewing the role of total IgG antibodies to food that such studies specifically measuring IgG4 are not included as they are a completely distinct entity and should be treated as such.**

One paper which highlights the difficulties of identifying reactions to dairy products is that of Hochwaller et al (2011). The diagnosis of food allergy based on the titration of specific IgG antibodies against food allergens is often performed when it is impossible to explain the patient's complaints using the classical methods of diagnosis of IgE and non-IgE-mediated food allergy, while the patient is convinced that it was caused by the consumed foods.

**The paper concluded:**

> ***"****The authors determined that there were no differences in sIgG levels in subclasses 1–4 between the patients intolerant to cow's milk protein and the patients who tolerated it. In addition, they noticed that only the patients with IgE-mediated allergy to milk had high levels of IgG1 and IgG4. IgG4 was higher in patients with an IgE-mediated allergy, whereas IgG2 and IgG3 were low in all studied groups. The patients intolerant to milk proteins had significantly lower levels of IgG compared to the patients with IgE-mediated allergy, and did not differ from persons tolerant to milk from the healthy group."*

This study highlights the unique and complex nature of milk allergy and intolerance. Non IgE reactions have been shown to include cellular responses not mediated by any class of antibody and lactose intolerance due to enzyme deficiencies, thus IgG specific antibodies alone are not necessarily diagnostic here, a fact that is often the case with specific IgE measurements.

## The Role of Antibodies According to Experts

Expert committees of international scientific societies have also spoken on the role of IgG antibodies in the diagnosis of food allergy. The European Academy of Allergology and Clinical Immunology (EACCI) issued a statement regarding food allergy, in which it stated that titrating IgG4 against foods is not recommended as a diagnostic tool. The presence of sIgG4 against foods indicates a repeated exposure to a food treated by the immune system as an alien protein and should not be treated as a sign of hypersensitivity but rather as a marker of immune tolerance associated with the activity of regulatory T cells. Specific IgG4 antibodies do not indicate food allergy or intolerance but a physiological response to the exposure to food. The International Consensus ON (ICON) document on food allergy, prepared under the aegis of EAACI as well as the American Academy of Allergy, Asthma & Immunology (AAAAI) and World Allergy Organization (WAO), clearly stresses that the titration of specific IgG4 against foods is not a recommended test in the diagnosis of food allergy. In the practical guidelines for food allergy dated November 2014, prepared by a group of experts from the American Academy of Allergy, Asthma & Immunology (AAAAI), the American College of Allergy, Asthma & Immunology (ACAAI), and the Joint Council of Allergy, Asthma & Immunology (JCAAI), it is stated that titrating allergen-specific IgG4 is not recommended in the diagnosis of non-IgE-mediated food allergies.

**Of note in these guidelines is the determination that allergen specific IgG4 assays (not total IgG 1-4) are not recommended for the routine evaluation of food allergies. As stated previously food specific IgG (total) antibodies as measured by the Food Detective product are not IgG4 specific nor is the test intended for the diagnosis of food allergy and therefore these statements do not apply to this product.**

An additional point to highlight is that while at first sight these position statements appear to represent evidence against the validity of food specific IgG testing i.e. total IgG, on a closer examination the statements made regarding IgG are either misinterpreted or are not referenced to

any published work and are simply the opinion of the authors. This in itself is sufficient reason for these statements to not be used against IgG testing.

A further example of the misuse of expert guidelines can be seen in the Guidelines for the Diagnosis and Management for Food Allergy in the United States (2010). Guideline 12 advises against using IgG for the routine evaluation of IgE-mediated food allergies only. Nowhere in the Guideline is it stated that IgG assays are not recommended for any other purpose. The overview section states that the "Guidelines focus on diseases that are defined as FA [food allergy] and include both immunoglobulin E (IgE)-mediated reactions to food and some non-IgE-mediated reactions to food." It does not address several areas of physiological responses to food, including IgG. This paper has often and inappropriately been used as a position paper on IgG food antibody testing. Whilst the guidelines recommended against using a number of tests, including allergen-specific IgG4 testing for the "routine" evaluation of food allergies. The guidelines do not conclude that IgG testing have no place in patient management

In addition to the inappropriateness of using the Guideline against IgG testing in general,

- The guidelines are not fixed protocols that must be followed.
- The document is not an official regulatory document of any government agency.

This document contains only a single reference to IgG under the heading of 'Non-standardised and unproven procedures'. Guideline 12: states 'The EP recommends not using any of the following non-standardized tests for the routine evaluation of IgE-mediated food allergy: Allergen-specific IgG4'. This is not referenced and a detailed review shows that out of 347 papers cited, there is not a single reference concerning the use of food specific IgG (total) measurements. Furthermore, the document also at no point makes any statement or reference to food specific IgG (total) measurements as being invalid or unproven.

Similarly, the European Academy of Allergy & Clinical Immunology (EAACI) Task Force Report 2008, cites a number of references, but it is interesting to note that the most recent relating to food IgG antibodies was 2006 and almost all papers were referring to IgG4 specifically. There was one specific reference to IgG (Wuthrich B, 1996), which is so obscure that PubMed cannot even show an abstract for it. The paper by Atkinson et al (2004) was also cited which actually makes the case for the clinical value of food specific IgG measurements in the management of IBS, however there was also a citation for Hunter JO. Food elimination IBS: the case for IgG testing remains doubtful. Gut 2005;54:1203, which whilst Dr Hunter is a recognised expert on IBS, was in fact a letter to the journal expressing a personal opinion.

This position statement is now some 8 years old and much of the evidence cited for the role of IgG4 has since been shown to be incorrect. Take for instance the statement "*Testing for IgG4 against foods is not recommended as a diagnostic tool."*

Recently (2015) the LEAP study was published in the New England Journal of Medicine

http://www.nejm.org/doi/full/10.1056/NEJMoa1414850#t=article

This was the ground-breaking findings of an international team of experts on the significant role IgG4 plays in the manifestation of peanut IgE mediated allergy. The results of these studies have radically changed our thinking of the development of food allergy and the role of immune tolerance. The crucial and diagnostic role that IgG4 measurements played in this research is cited throughout the report. These new findings have also helped to clarify the important difference between IgG (total) and IgG4

specific measurements. When discussing IgG (total) as a tool for assessing food intolerance or sensitivity IgG4 measurements are inappropriate and should not be included.

## If the position statements from the American and European societies are out of date what about the most recent statements?

The publication of 'Diagnosis and management of food allergy' (Elissa M. Abrams MD, Scott H. Sicherer MD) in the Canadian Medical Association Journal in October 2016, made the following statement:

> *"Food-specific immunoglobulin G (IgG) testing is being increasingly used to identify food "sensitivities." This testing has not been validated nor supported by research."*

Again, there was not a single paper cited to back this statement, instead the paper cited the previous statements made by the EAACI above in 2008, which effectively means that this statement has been made without reference to any published work.

Additional potential arguments against IgG include the following statement:

> *"IgG subclasses indicate an antibody is present but these levels are not diagnostic of a disease process."*

This statement implies that that a laboratory test must be diagnostic of a disease process. Many laboratory tests are not: IgE, the test recommended for food allergy testing, is not by itself diagnostic of food allergies. Guideline 7 recommends allergen-specific serum IgE tests to identify foods that potentially provoke IgE-mediated food-induced allergic reactions, but states that by themselves these tests are not diagnostic of food allergy. Therapeutic drug monitoring, drug screening, sex hormone levels, full blood count, biochemistry screens and even cholesterol levels are other examples of widely used tests that are not diagnostic of disease.

**In summary:**

These guidelines were written for IgE-mediated type I) food reactions and a few, limited non-IgE-mediated reactions to foods including IgG4. They do not attempt to address other types of reactions to foods, including type III, which is related to IgG (total) antibody testing.

- The Guidelines were not intended to be used for regulatory purposes; they do, in fact, state that clinical judgment remains paramount.
- There are little or no peer reviewed published works regarding the lack of validity or efficacy of food specific IgG (total) antibody assessments to back these statements.
- These statements are out of date and in need of review and update.
- There is substantial scientific and clinical support for the benefit of IgG (total) food antibody testing in patient care.
- A test does not need to be diagnostic to be clinically relevant and useful.

Below is a list of references to several peer-reviewed documents that support the use of serological IgG (total) food antibody tests for use in patients that we believe constitutes strong evidence to support the role of IgG in food intolerance testing.

It also includes references specifically citing food specific IgG antibody measurements which make the statement that there is no link between IgG antibodies and food intolerance. Scientific Reasons have been outlined as to why these references are not applicable.

## Evidence-Based Clinical Relevance of Food Specific Serum IgG Antibodies

It has been proposed that food-specific IgG antibodies are involved in the delayed, type III hypersensitivity reaction. This mechanism does not utilise IgE. Pathologic consequences stem from the formation of IgG immune complexes and initiation of the complement system.

A growing body of medical literature supports the clinical value of measuring food-specific IgG antibodies to guide therapeutic dietary changes. A number of studies involve IBS patients. These are cited below with full abstracts. (Atkinson 2004, Sheldon et al. 2004; Drisko 2006, Bischoff et al. 2006; Yang and Li 2007; Zuo, Li et al. 2007; Ou-Yang, You et al. 2008) In all studies, significant clinical improvement was gained by using IgG (total) testing to screen for foods for dietary exclusion.

Irritable Bowel Syndrome is estimated to occur in in12% to 22% of the UK population and is a disorder of high direct and indirect medical costs (Mertz 2003). Any improved treatment and management would be of significant benefit not only to patient outcome, but also to the reduction in health care costs. In a review of all published literature from 1966 to 2015 relating to IBS, a report in the World Journal of Gastroenterology in 2015, (Food allergy in irritable bowel syndrome: The case of non-celiac wheat sensitivity. Mansueto P, D'Alcamo A, Seidita A, Carroccio A. World J Gastroenterol. 2015 June 21;21(23):7089-109. Review.) concluded that hypersensitivity reactions may play a role in causing IBS symptoms in a subset of patients. Furthermore, the increase of food-specific IgG titres could be a specific reaction, rather than a non-specific response to increased gut mucosal permeability. The authors concluded that 'Pending further scientific evidence, the concept of food allergy (adverse food reactions) should be included as a possible cause of IBS, and a dietary approach may have a place in the routine clinical management of IBS'.

Other studies looking at a variety of conditions showed IgG testing to be clinically useful in ameliorating symptoms include Dixon 2000, Wilders-Truschnig 2008, Mangge et al. 2008; Alpay, Ertas et al. 2010).

## Study Using Cambridge Nutritional Sciences IgG ELISA

### FOOD ELIMINATION AS TREATMENT FOR PRIMARY HEADACHE IN CHILDREN (2016)

Sepideh Taheri, Irfaan Cader, Jamie Seabrook, Elizabeth Mazza, Margo De Vries, Craig Campbell
1. Department of Paediatrics, Western University, London, Ontario, Canada
2. School of Food and Nutritional Sciences, Brescia University College at Western University, London, Ontario, Canada

### BACKGROUND

Chronic recurrent headache (HA) is a significant cause of morbidity in children, with a prevalence of 10% being recorded in 3-14 year olds and evidence of global increase in incidence.

Dietary factors are considered to play a significant role in the aetiology of recurrent HA in adults; however, good data to confirm this statement in the paediatric population is lacking.

1. Evidence in adults demonstrates that HAs and other conditions may be secondary to an immunological mechanism mediated by immunoglobulin G (IgG) against certain foods. (Alpay et al 2009))

Exclusion of foods based on high serum IgG levels has led to significant reduction of symptoms in adults; however, this has yet to be studied in children.

**STUDY DESIGN AND METHODOLOGY**

**DESIGN:** Randomized, controlled, single-centre trial.

**SETTING:** Outpatient tertiary paediatric HA clinic, Children's Hospital, London, Ontario.

**PARTICIPANTS**: 50 children aged 7-15 referred to the HA clinic.

EXCLUSION CRITERIA: 1) Children less than 7 years of age. 2) Secondary HA. 3) Concomitant use of complementary and alternative medication. 4) Elective surgery planned for within 24 weeks of the start of the study. 5) Diagnosis of failure-to-thrive. 6) Children with body weight plotting lower than 5th centile on a growth chart. 7) Medical conditions that in the opinion of the PI would be unsafe for trial participation. 8) Lack of follow-up or failure to comply with study procedures.

**METHODOLOGY:**

Patients were randomized to either conventional or dietary intervention group in the ratio of 1:1.

The conventional group received standard treatment for HA. The dietary intervention group receive targeted dietary elimination advice based on serum IgG positivity and/or non-IgG foods, based on frequency of consumption from their food diary.

Only 1 IgG- positive food was eliminated in each 6-week visit. Handouts were given to provide alternative recipe ideas. In non-reactive patients, non-IgG foods (e.g. caffeine, MSG) are eliminated.

Patients were followed up at 6-weekly intervals for a total of 5 study visits (24 weeks). Non-responders in each group were crossed over to the other arm of the study. Food and HA diaries were reviewed.

IgG levels were measured at start and end of study using Food IgG Screen Kits (120 foods) provided by Cambridge Nutritional Sciences, and were analysed using the ELISA technique.

**RESULTS**

11 patients in diet group resolved (HA reduction >90%)

2 patients in diet group improved (HA reduction by 50-90%)

7 patients crossed study arms (6 patients from conventional to dietary and 1 from dietary to conventional due to non-response)

1 patient had no food triggers identified and remained in conventional group

**Table1: Result of dietary intervention on IgG levels.**

| Dietary Intervention | IgG Level (%) | | Δ |
|---|---|---|---|
| | Before | After | |
| Cow's Milk | 190 | 106 | -84% |
| Egg White | 121 | 97 | -25% |
| Goat/Sheep Milk | 116 | 54 | -62% |
| Gluten | 103 | 88 | -16% |

This table displays the average relative change in IgG levels after eliminating a certain food for the period of the study. Values are represented as a percentage relative to the minimum IgG threshold required to indicate a strong positive reaction.

**The study concluded:**

Elimination of 1 or 2 foods, based on IgG positivity, has resulted in significant reduction in HA frequency and severity in children.

This correlates well with a demonstrated reduction in serum IgG levels at the end versus beginning of the study.

Further investigation is required to clarify the correlation between IgG-mediated food sensitivity and primary HA in children.

## Commonly Quoted Reasons to Disregard Food Sensitivity Tests

### *1. IgG Test Lack Clinical Validity:*

***The problem is the IgG antibody is the most abundant antibody in the body and is not clinically validated for diagnosing food Allergy.***

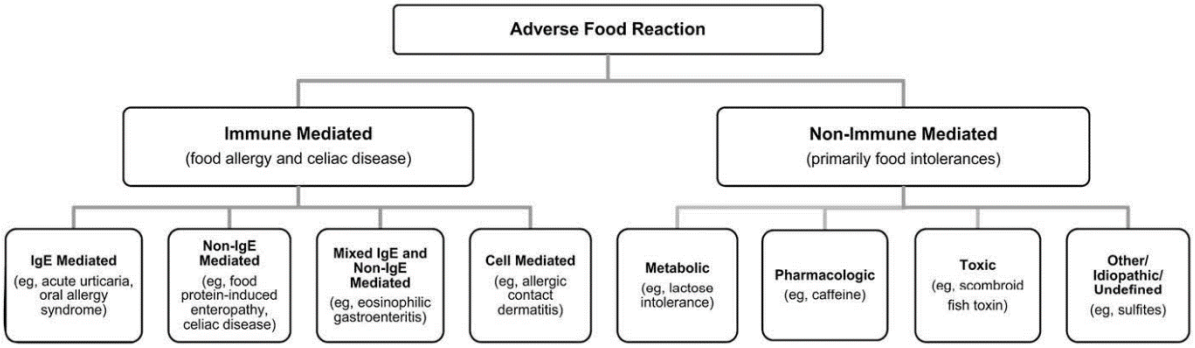
RESPONSE

The collective position of various allergy societies around the world, until recently has been that 'Testing for IgG4 against foods is not recommended as a diagnostic tool'.

It is very important to understand that these societies have been established for many years, when our knowledge of allergy and hypersensitivity was in its infancy as such they are only concerned with allergy in the form of IgE mediated reactions and some forms of cellular responses. They have no interest nor expertise in IgG mediated food intolerance.

The statement that 'IgG measurements have no value in the diagnosis of food allergy' strictly refers to the role of IgG4, this is a unique molecule that has a very important role to play in the development of true food allergy (IgE) and plays no part in the assessment of food sensitivity/intolerance

With regards to total IgG measurements such as ours for the identification of food intolerance or sensitivity, this is beyond the remit of these allergy societies and since they refer only to IgG4 they in fact they neither endorse or do not endorse such testing.




*Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel in 2010. Types of adverse reactions to food, IgG mediated reactions are not included.*

For example, Guideline 12: The EP recommends not using any of the following no standardised tests for the routine evaluation of IgE-mediated Food Allergy:

- Basophil histamine release/activation
- Lymphocyte stimulation
- Facial thermography
- Gastric juice analysis
- Endoscopic allergen provocation
- Hair analysis

- Applied kinesiology
- Provocation neutralization
- Allergen-specific $IgG_4$
- Cytotoxicity assays
- Electrodermal test (Vega)
- Mediator release assay (LEAP diet)

They refer specifically to IgG4 measurements and specifically for the routine evaluation of IgE-mediated Food Allergy, indeed there is no mention or references in this document refereeing to IgG mediated food intolerance or sensitivity.

There is a vast amount of publications, demonstrating the utility of such measurements in a variety of conditions.

***Having a positive IgG response to certain foods does not mean that your body is reacting negatively against this food, instead, it demonstrates tolerance.***

RESPONSE

This statement is again about IgG4 specifically, where in this instance the statement is essentially true, however where Total IgG is concerned elevated levels do no indicate tolerance, in fact elevated levels by definition demonstrate that the immune system has been stimulated to produce an inappropriate reaction to the food we are consuming and actually reflects a breakdown in tolerance.

***2. These test exploit consumer trust; without solid research to back up claims.***

RESPONSE

There is an ever-growing body of published evidence demonstrating the clinical utility of food IgG antibody testing, these include but are not limited to the following:

Food allergy in irritable bowel syndrome: The case of non-celiac wheat sensitivity

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4476871/

Serological Investigation of Food Specific Immunoglobulin G Antibodies in Patients with Inflammatory Bowel Diseases

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0112154

Clinical relevance of IgG antibodies against food antigens in Crohn's disease: a double-blind cross-over diet intervention study.

https://www.ncbi.nlm.nih.gov/pubmed/20130407

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

https://www.thieme-connect.com/DOI/DOI?10.1055/s-2007-993165

Diet restriction in migraine, based on IgG against foods: A clinical double-blind, randomised, cross-over trial

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2899772/

IgG-based elimination diet in migraine plus irritable bowel syndrome.

https://www.ncbi.nlm.nih.gov/pubmed/23216231

Testing for food reactions: the good, the bad, and the ugly.

https://www.ncbi.nlm.nih.gov/pubmed/20413700

### *3. Lack of Symptom Improvement*

RESPONSE

There is a wealth of published literature demonstrating the effectiveness of exclusion diets guided by total IgG assessments in alleviating a range of symptoms. A resource document containing all such abstracts for these papers is available from Cambridge Nutritional Sciences).

### *4. Unnecessary Dietary Restriction*

RESPONSE

It is very important that any assessment of food reactions including those of total IgG food sensitivities should include advice from a suitably qualified professional to ensure that any diet to be followed is nutritionally sound and appropriate, all reputable testing laboratories will recommend this, support materials for professionals are available from Cambridge Nutritional Sciences.

### *5. Health information and dietary advice is flying at consumers from all directions. But not all this information is true or science-based and not everyone is a trusted authority.*

RESPONSE

This is very true, equally there is unfortunately a lot of articles written about this complex issue from individuals who are not suitably qualified nor recognised experts on the subject of allergy, hypersensitivity and intolerance. Any such reviews in any media on this subject should be balanced, not prejudiced and most importantly using the most up to date information available not simply repeating old and out of date statements.

# References to Support IgG Testing

**Agarwal S, Mayer L. Mucosal immunity. In: Food Allergy: Adverse Reactions to Foods and Food Additives. 4th edition. Metcalfe DD, Sampson HA, Simon RA (eds). Blackwell Publishing, Oxford 2008; 19-29.**

**Alpay, K., M. Ertas, et al. (2010). "Diet restriction in migraine, based on IgG against foods: a clinical double-blind, randomised, cross-over trial." Cephalalgia: an international journal of headache 30(7): 829-837.**

INTRODUCTION: It is well-known that specific foods trigger migraine attacks in some patients. We aimed to investigate the effect of diet restriction, based on IgG antibodies against food antigens on the course of migraine attacks in this randomised, double blind, cross-over, headache-diary based trial on 30 patients diagnosed with migraine without aura.

METHODS: Following a 6-week baseline, IgG antibodies against 266 food antigens were detected by ELISA. Then, the patients were randomised to a 6-week diet either excluding or including specific foods with raised IgG antibodies, individually. Following a 2-week diet-free interval after the first diet period, the same patients were given the opposite 6-week diet (provocation diet following elimination diet or vice versa). Patients and their physicians were blinded to IgG test results and the type of diet (provocation or elimination). Primary parameters were number of headache days and migraine attack count. Of 30 patients, 28 were female and 2 were male, aged 19-52 years (mean, 35 +/- 10 years).

RESULTS: The average count of reactions with abnormally high titre was 24 +/- 11 against 266 foods. Compared to baseline, there was a statistically significant reduction in the number of headache days (from 10.5 +/- 4.4 to 7.5 +/- 3.7; P < 0.001) and number of migraine attacks (from 9.0 +/- 4.4 to 6.2 +/- 3.8; P < 0.001) in the elimination diet period.

**CONCLUSION:** This is the first randomised, cross-over study in migraineurs, showing that diet restriction based on IgG antibodies is an effective strategy in reducing the frequency of migraine attacks.

**Aydinlar, E. I., Dikmen, P. Y., Tiftikci, A., Saruc, M., Aksu, M., Gunsoy, H. G. and Tozun, N. (2013), IgG-Based Elimination Diet in Migraine Plus Irritable Bowel Syndrome. Headache: The Journal of Head and Face Pain, 53: 514–525.**

OBJECTIVES: To evaluate therapeutic potential of the immunoglobulin G (IgG)-based elimination diet among migraine patients with irritable bowel syndrome (IBS).

BACKGROUND: Food elimination has been suggested as an effective and inexpensive therapeutic strategy in patients with migraine and concomitant IBS in the past studies.

METHODS: A total of 21 patients (mean [standard deviation] age: 38.0 [11.2] years; 85.7% females) diagnosed with migraine and IBS were included in this double-blind, randomized, controlled, cross-over clinical trial composed of baseline (usual diet), first diet (elimination or provocation diets), and second diet (interchange of elimination or provocations diets) phases and 4 visits.

RESULTS: IgG antibody tests against 270 food antigens revealed mean (standard deviation) reaction count to be 23.1 (14.1). Compared with baseline levels, elimination diet per se was associated with significant reductions in attack count (4.8 [2.1] vs 2.7 [2.0]; P < .001), maximum attack duration (2.6 [0.6] vs. 1.4 [1.1] days; P < .001), mean attack duration (1.8 [0.5] vs. 1.1 [0.8] days; P < .01), maximum

attack severity (visual analog scale 8.5 [1.4] vs. visual analog scale 6.6 [3.3]; P < .001), and number of attacks with acute medication (4.0 [1.5] vs. 1.9 [1.8]; P < .001). There was a significant reduction in pain-bloating severity (1.8 [1.3] vs. 3.2 [0.8]; P < .05), pain-bloating within the last 10 days (3.2 [2.8] vs. 5.5 [3.1]; P < .05), and improvement obtained in quality of life (3.6 [1.4] vs. 2.9 [1.0]; P < .05) by the elimination diet as compared with provocation diet.

**CONCLUSION:** Our findings indicate that food elimination based on IgG antibodies in migraine patients who suffer from concomitant IBS may effectively reduce symptoms from both disorders with possible positive impact on the quality of life of the patients as well as potential savings to the health-care system.

**Atkinson, W., T. A. Sheldon, et al. (2004). "Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial." Gut 53(10): 1459-1464.**

BACKGROUND: Patients with irritable bowel syndrome (IBS) often feel they have some form of dietary intolerance and frequently try exclusion diets. Tests attempting to predict food sensitivity in IBS have been disappointing but none has utilised IgG antibodies.

AIMS: To assess the therapeutic potential of dietary elimination based on the presence of IgG antibodies to food.

PATIENTS: A total of 150 outpatients with IBS were randomised to receive, for three months, either a diet excluding all foods to which they had raised IgG antibodies (enzyme linked immunosorbant assay test) or a sham diet excluding the same number of foods but not those to which they had antibodies.

METHODS: Primary outcome measures were change in IBS symptom severity and global rating scores. Non-colonic symptomatology, quality of life, and anxiety/depression were secondary outcomes. Intention to treat analysis was undertaken using a generalised linear model.

RESULTS: After 12 weeks, the true diet resulted in a 10% greater reduction in symptom score than the sham diet (mean difference 39 (95% confidence intervals (CI) 5-72); p = 0.024) with this value increasing to 26% in fully compliant patients (difference 98 (95% CI 52-144); p<0.001). Global rating also significantly improved in the true diet group as a whole (p = 0.048, NNT = 9) and even more in compliant patients (p = 0.006, NNT = 2.5). All other outcomes showed trends favouring the true diet. Relaxing the diet led to a 24% greater deterioration in symptoms in those on the true diet (difference 52 (95% CI 18-88); p = 0.003).

**CONCLUSION:** Food elimination based on IgG antibodies may be effective in reducing IBS symptoms and is worthy of further biomedical research.

**Bentz S, Hausmann M, Piberger H, Kellermeier S, Paul S, Held L, Falk W, Obermeier F, Fried M, Schölmerich J, Rogler G, Clinical Relevance of IgG Antibodies against Food Antigens in Crohn's Disease: A Double-Blind Cross-Over Diet Intervention Study. Digestion 2010;81:252-264**

BACKGROUND: Environmental factors are thought to play an important role in the development of Crohn's disease (CD). Immune responses against auto-antigens or food antigens may be a reason for the perpetuation of inflammation.

METHODS: In a pilot study, 79 CD patients and 20 healthy controls were examined for food immunoglobulin G (IgG). Thereafter, the clinical relevance of these food IgG antibodies was assessed in a double-blind cross-over study with 40 patients. Based on the IgG antibodies, a nutritional intervention was planned. The interferon (IFN) gamma secretion of T cells was measured. Eosinophil-derived neurotoxin was quantified in stool.

RESULTS: The pilot study resulted in a significant difference of IgG antibodies in serum between CD patients and healthy controls. In 84 and 83% of the patients, respectively, IgG antibodies against processed cheese and yeast were detected. The daily stool frequency significantly decreased by 11% during a specific diet compared with a sham diet. Abdominal pain reduced and general well-being improved. IFN gamma secretion of T cells increased. No difference for eosinophil-derived neurotoxin in stool was detected.

**CONCLUSION:** A nutritional intervention based on circulating IgG antibodies against food antigens showed effects with respect to stool frequency. The mechanisms by which IgG antibodies might contribute to disease activity remain to be elucidated.

**Cai C, Shen J, Zhao D, Qiao Y, Xu A, Jin S, et al. (2014) Serological Investigation of Food Specific Immunoglobulin G Antibodies in Patients with Inflammatory Bowel Diseases. PLoS ONE 9(11): e112154.**

OBJECTIVE: Dietary factors have been indicated to influence the pathogenesis and nature course of inflammatory bowel diseases (IBD) with their wide variances. The aim of the study was to assess the prevalence and clinical significance of 14 serum food specific immunoglobulin G (sIgG) antibodies in patients with IBD.

METHODS: This retrospective study comprised a total of 112 patients with IBD, including 79 with Crohn's disease (CD) and 33 with ulcerative colitis (UC). Medical records, clinical data and laboratory results were collected for analysis. Serum IgG antibodies against 14 unique food allergens were detected by semi-quantitative enzyme linked immunosorbent assay (ELISA).

RESULTS: Food sIgG antibodies were detected in 75.9% (60/79) of CD patients, 63.6% (21/33) of UC patients and 33.1% (88/266) of healthy controls (HC). IBD patients showed the significantly higher antibodies prevalence than healthy controls (CD vs. HC, P = 0.000; UC vs. HC, P = 0.001). However no marked difference was observed between CD and UC groups (P = 0.184). More subjects were found with sensitivity to multiple antigens (≥3) in IBD than in HC group (33.9% vs.0.8%, P = 0.000). Egg was the most prevalent food allergen. There was a remarkable difference in the levels of general serum IgM (P = 0.045) and IgG (P = 0.041) between patients with positive and negative sIgG antibodies. Patients with multiple positive allergens (≥3) were especially found with significant higher total IgG levels compared with sIgG-negative patients (P = 0.003). Age was suggested as a protective factor against the occurrence of sIgG antibodies (P = 0.002).

**CONCLUSION:** The study demonstrates a high prevalence of serum IgG antibodies to specific food allergens in patients with IBD. sIgG antibodies may potentially indicate disease status in clinical and be utilized to guide diets for patients.

**Dixon, H. S. (2000). "Treatment of delayed food allergy based on specific immunoglobulin G RAST testing." Otolaryngology--head and neck surgery: official journal of American Academy of Otolaryngology-Head and Neck Surgery 123(1 Pt 1): 48-54.**

This preliminary, descriptive study after extensive clinical experience demonstrates specific IgG food RASTs done in 114 consecutive patients with strong positive histories for delayed food allergy. Elimination of the positive foods was the sole means of treatment. The symptoms leading to the test are detailed, and the method of workup is reviewed. The overall results demonstrated a 71% success rate for all symptoms achieving at least a 75% improvement level. Of particular interest was the group of patients with chronic, disabling symptoms, unresponsive to other intensive treatments. Whereas 70% obtained 75% or more improvement, 20% of these patients obtained 100% relief.

**Drisko, J., B. Bischoff, et al. (2006). "Treating irritable bowel syndrome with a food elimination diet followed by food challenge and probiotics." Journal of the American College of Nutrition 25(6): 514-522.**

OBJECTIVE: In Irritable Bowel Syndrome, the gut-associated immune system may be up-regulated resulting in immune complex production, low-grade inflammation, loss of Class I bacteria, and translocation of inflammatory mediators and macromolecules outside of the GI lumen. Since food intolerance may be one of the reasons for this upregulation, our goal was to investigate the role of food intolerance in IBS patients.

METHODS: In this open label pilot study, we enrolled 20 patients with IBS by Rome II criteria (15 women, ages 24-81) who had failed standard medical therapies in a tertiary care GI clinic. Baseline serum IgE and IgG food and mold panels, and comprehensive stool analysis (CSA) were performed. Breath-hydrogen testing and IBS Quality-of-Life (QOL) questionnaires were obtained. Patients underwent food elimination diets based on the results of food and mold panels followed by controlled food challenge. Probiotics were also introduced. Repeat testing was performed at 6-months. We followed up with this cohort at 1 year after trial completion to assess the reported intervention and for placebo effect.

RESULTS: Baseline abnormalities were identified on serum IgG food and mold panels in 100% of the study subjects with significant improvement after food elimination and rotation diet ($p < 0.05$). Significant improvements were seen in stool frequency ($p < 0.05$), pain ($p < 0.05$), and IBS-QOL scores ($p < 0.0001$). Imbalances of beneficial flora and dysbiotic flora were identified in 100% of subjects by CSA. There was a trend to improvement of beneficial flora after treatment but no change in dysbiotic flora. The 1-year follow up demonstrated significant continued adherence to the food rotation diet (4.00 +/- 1.45), minimal symptomatic problems with IBS (4.00 +/- 1.17), and perception of control over IBS (4.15 +/- 1.23). The continued use of probiotics was considered less helpful (3.40 +/- 1.60).

**CONCLUSION:** These data demonstrate that identifying and appropriately addressing food sensitivity in IBS patients not previously responding to standard therapy results in a sustained clinical response and impacts on overall wellbeing and quality of life in this challenging entity.

**Gell PGH, Coombs RRA, eds. Clinical Aspects of Immunology. 1st ed. Oxford, England: Blackwell; 1963. Section IV, Chapter 1**

**Geoffrey Hardman, Gillian Hart, "Dietary advice based on food-specific IgG results", Nutrition & Food Science, 2007 Vol. 37 Iss: 1, pp.16 - 23**

PURPOSE: To provide evidence that elimination diet based on food-specific IgG test results is an effective, reliable and valid aid to the management of chronic medical conditions. Design/methodology/approach – A postal survey, commissioned by Allergy UK, was carried out with 5,286 subjects reporting a wide range of chronic medical conditions, who had taken a food-specific IgG enzyme-linked immunosorbant assay blood test. Questionnaires, issued three months after the results, were analysed to investigate the effect of eliminating the foods identified by the test. To check for response bias, a separate group of patients who had not responded were interviewed by telephone. The analysis and reporting of the data was carried out at the University of York. Findings – Of patients who rigorously followed the diet 75.8 per cent had a noticeable improvement in their condition. Of patients who benefited from following the recommendations 68.2 per cent felt the benefit within three weeks. Those who reported more than one condition were more likely to report noticeable improvement. 81.5 per cent of those that dieted rigorously and reported three or more co-morbidities showed noticeable improvement in their condition. For those who dieted rigorously and reported high benefit, 92.3 per cent noticed a return of symptoms on reintroduction of the offending foods.

**Originality/value –** These data provide evidence for the use of elimination diet based on food-specific IgG blood test results as an aid to management of the symptoms of a range of chronic medical conditions.

**Mansueto, Pasquale et al. "Food Allergy in Irritable Bowel Syndrome: The Case of Non-Celiac Wheat Sensitivity." World Journal of Gastroenterology: WJG 21.23 (2015): 7089–7109. Web. 19 Oct. 2016.**

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders, having a prevalence of 12%-30% in the general population. Most patients with IBS attribute their symptoms to adverse food reactions. We review the role of diet in the pathogenesis of IBS and the importance of dietary factors in the management of these patients. The MEDLINE electronic database (1966 to Jan 2015) was searched using the following keywords: "food", "diet", "food allergy", "food hypersensitivity", "food intolerance", "IBS", "epidemiology", "pathogenesis", "pathophysiology", "diagnosis", "treatment". We found 153 eligible papers; 80 were excluded because: not written in English, exclusive biochemical and experimental research, case reports, reviews, and research otherwise not relevant to our specific interest. We selected 73 papers: 43 original papers, 26 reviews and 4 letters to the editor. These papers focused on IBS pathogenesis, the association between IBS and atopy, and between IBS and food allergy, the relationship between IBS and non-celiac wheat sensitivity, the role of diet in IBS.

***Pending further scientific evidence, a cautious approach is advisable but the concept of food allergy should be included as a possible cause of IBS, and a dietary approach may have a place in the routine clinical management of IBS.***

***Irritable bowel syndrome (IBS) is one of the most common GI disorders, having a prevalence of 12%-30%.***

***Most patients with IBS attribute their symptoms to adverse food reactions.***

***Studies reported that serum IgG levels are higher in patients with IBS and food allergy history, perhaps related to an inflamed or "leaky" gut.***

***Hypersensitivity reactions may play a role in causing IBS symptoms in a subset of patients.***

***Patients might have selective gut permeability to food antigens. The increase of food-specific IgG titers could be a specific reaction, rather than a non-specific response to increased gut mucosal permeability.***

**Mertz, H. R. (2003). "Irritable bowel syndrome." The New England journal of medicine 349(22): 2136-2146. Ou-Yang, W. X., J. Y. You, et al. (2008). "[Application of food allergens specific IgG antibody detection in chronic diarrhea in children]." Zhongguo dang dai er ke za zhi = Chinese journal of contemporary pediatrics 10(1): 21-24.**

OBJECTIVE: The causes of chronic diarrhea in children are complex. At present, food allergy is generally viewed as an important cause of this disorder, and IgG-mediated delayed allergy plays a major role in this process. This study aimed to explore the link between food specific IgG and chronic diarrhea in children, as well as the value of food antigens-specific IgG antibody detection in the management of this disorder.

METHODS: Eighty-two children with chronic diarrhea and 30 healthy controls were enrolled. Serum levels of specific IgG antibody to 14 kinds of food were detected using ELISA. The results were classified into four grades: Grade 0 (negative), Grade 1 (mild allergy), Grade 2 (moderate allergy) and Grade 3 (severe allergy). The patients received a diet treatment based on the results of food specific IgG antibody detection. Children with negative IgG antibody were allowed to continue their current diet. In children with Grade 1 allergy, the food responsible for the IgG antibody positive test was given only at an interval of four days. In children with Grade 2 or 3, the offending food was eliminated from the diet.

RESULTS: Of the 82 children with chronic diarrhea, 79 (96.2%) had increased specific IgG levels for one or more of the 14 foods tested compared to 8 (26.7%) of the controls (P <0.01). The majority of patients showed increased specific IgG levels for milk (68.3%) and egg (62.2%). A low proportion of patients (2.4%) was allergic to chicken, and no patient was allergic to pork. The symptoms were improved in 65 patients (79.3%) after 1 week to 3 months of diet treatment.

**CONCLUSION:** Food allergy is one of major causes of chronic childhood diarrhea. Food specific IgG antibody detection may assist in the dietary management of this disorder.

**Mullin GE1, Swift KM, Lipski L, Turnbull LK, Rampertab SD. Testing for Food Reactions: The Good, the Bad, and the Ugly. Nutr Clin Pract April 2010 25: 192-198,**

An increasing number of commercial tests for food allergies are marketed to consumers and healthcare practitioners with tenuous claims. The aim of this article is to provide an evidence-based review of the tests and procedures that currently are used for patients with suspected food allergy. A systematic review of the literature evaluating the validity of tests and procedures used in food reactions was performed using conventional search engines (eg, PubMed, Ovid) as well as consumer sites (eg, Google, Bing). The National Library of Medicine Medical Subject Headings (MeSH) term food hypersensitivity was used along with food allergy testing, food sensitivity testing, food intolerance testing, and adverse food reactions. Of the results obtained, testing for immunoglobulin E (IgE)-mediated food allergy was best represented in PubMed. IgE-based testing continues to be the gold standard for suspected food allergies. Among modalities used by many conventional and alternative

practitioners, immunoglobulin G (IgG)-based testing showed promise, with clinically meaningful results. It has been proven useful as a guide for elimination diets, with clinical impact for a variety of diseases. Mediator release testing and antigen leukocyte cellular antibody testing were only represented on consumer sites.

**CONCLUSION:** Further investigation into the validity and the clinical application of these tests and procedures is required. Disclosing the basis for food reactions continues to present a diagnostic challenge, and testing for food allergies in the context of an appropriate clinical history is paramount to making the correct diagnosis.

**PAGANELLI, R. J. LEVINSKY & D. J. ATHERTON. Detection of specific antigen within circulating immune complexes: validation of the assay and its application to food antigen-antibody complexes formed in healthy and food-allergic subjects. Clin. exp. Immunol. (1981) 46, 44-53**

BACKGROUND: A simple two-step method for the detection of specific antigen within immune complexes is described. The immune complexes are precipitated from serum by polyethylene glycol, dissociated by incubation in acid pH buffer and adsorbed onto the surface of polystyrene tubes. The antigen is detected by the binding of a radiolabelled affinity-purified specific antibody. The assay can detect the antigen within both antigen- and antibody-excess immune complexes of any immunoglobulin class, and can also allow semiquantitative comparison of different samples. Immune complexes containing food protein antigens after eating have been found in the serum of both normal subjects and atopic patients; the latter group showed higher mean levels of antigen-specific immune complexes. The method can be adopted for large-scale screening of clinical samples for suspected antigens if suitable antisera are available.

**Wilders-Truschnig, M., H. Mangge, et al. (2008). "IgG antibodies against food antigens are correlated with inflammation and intima media thickness in obese juveniles." Experimental and clinical endocrinology & diabetes: official journal, German Society of Endocrinology [and] German Diabetes Association 116(4): 241-245.**

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.

**CONCLUSION:** Abnormal immune reactions mediated by IgG antibodies coexisted in patients with IBS. It is of great significance in treating IBS by eliminating the allergic foods according to the serum level of food-specific IgG antibodies.

*"Dietary elimination therapy based on the presence of IgG antibodies to food components may be indicated. Such dietary therapy may be effective in reducing low grade inflammation and thereby preventing clinical consequences like type 2 diabetes and atherogenesis."*

**Yang, C. M. and Y. Q. Li (2007). "[The therapeutic effects of eliminating allergic foods according to food-specific IgG antibodies in irritable bowel syndrome]." Zhonghua nei ke za zhi [Chinese journal of internal medicine] 46(8): 641-643.**

OBJECTIVE: To explore the therapeutic effects on irritable bowel syndrome (IBS) by eliminating the allergic foods according to food-specific IgG antibodies and to clarify the etiopathological role and mechanism of food allergy.

METHODS: The food-specific IgG antibodies to a panel of 14 different food antigens in serum were detected with ELISA in fifty-five cases with diarrhea-dominant IBS, thirty-two with constipation-dominant IBS and eighteen normal controls. The frequency and severity index of symptoms and scores of Irritable Bowel Syndrome Quality of Life (IBS-QOL) in thirty-five cases with positive food specific IgG were observed before and after elimination of allergic foods for two months.

RESULTS: The positive rate of serum food-specific IgG antibodies was 63.6 percent in patients with diarrhea-dominant IBS and 43.8 percent in constipation-dominant IBS. Both were higher than that in normal controls. After eliminating allergic foods for four weeks according to the levels of serum food-specific IgG antibodies, the frequency of symptoms decreased from (3.79 +/- 1.58) to (1.67 +/- 0.70) per week and the severity from 3.18 +/- 1.46 to 1.52 +/- 0.67 with significant differences. After eight weeks, the frequency of symptoms decreased from (3.79 +/- 1.58) to (1.53 +/- 0.69) per week and the severity from 3.18 +/- 1.46 to 1.45 +/- 0.66, also with significant differences. After eliminating allergic foods, the overall health score and the eight dimensionality integrals of QOL except avoiding food in patients with IBS increased significantly than those before treatment. At the end of eight weeks, the symptoms relieved completely in 31.4 percent of the cases and remarkably in 34.3 percent.

**CONCLUSION:** Abnormal immune reactions mediated by IgG antibodies coexisted in patients with IBS. It is of great significance in treating IBS by eliminating the allergic foods according to the serum level of food-specific IgG antibodies.

**Zuo, X. L., Y. Q. Li, et al. (2007). "Alterations of food antigen-specific serum immunoglobulins G and E antibodies in patients with irritable bowel syndrome and functional dyspepsia." Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology 37(6): 823-830.**

BACKGROUND: Post-prandial worsening of symptoms as well as adverse reactions to one or more foods are common in the patients with functional gastrointestinal diseases, such as irritable bowel syndrome (IBS) and functional dyspepsia (FD). However, the role played by true food allergy in the

pathogenesis of these diseases is still controversial and there are no well-established tests to identify food allergy in this condition.

OBJECTIVE: To investigate serum food antigen-specific IgG, IgE antibody and total IgE antibody titres in controls and patients with IBS and FD, and to correlate symptoms with the food antigen-specific IgG titres in IBS and FD patients.

METHODS: Thirty-seven IBS patients, 28 FD patients and 20 healthy controls participated in this study. Serum IgG and IgE antibody titres to 14 common foods including beef, chicken, codfish, corn, crab, eggs, mushroom, milk, pork, rice, shrimp, soybean, tomatoes and wheat were analysed by ELISA. Serum total IgE titres were also measured. Last, symptomatology was assessed in the study.

RESULTS: IBS patients had significantly higher titres of IgG antibody to crab (P=0.000), egg (P=0.000), shrimp (P=0.000), soybean (P=0.017) and wheat (P=0.004) than controls. FD patients had significantly higher titres of IgG antibody to egg (P=0.000) and soybean (P=0.017) than controls. The percentage of individuals with detectable positive food antigen-specific IgE antibodies of the three groups did not show any significant differences (P=0.971). There were no significant differences between IBS patients, FD patients and controls in the serum total IgE antibody titres (P=0.978). Lastly, no significant correlation was seen between symptom severity and serum food antigen specific IgG antibody titres both in IBS and FD patients.

**CONCLUSION:** Serum IgG antibody titres to some common foods increased in IBS and FD patients compared to controls. But there is no significant correlation between symptom severity and elevated serum food antigen-specific IgG antibodies in these patients.

## References Against IgG Testing

**Antico A, Pagani M, Vescovi PP, et al. Food-specific IgG4 lack diagnostic value in adult patients with chronic urticaria and other suspected allergy skin symptoms. Int Arch Allergy Immunol 2011; 155: 52-6.**

BACKGROUND: Specific IgG4 dosing against food is proposed to the public by a lot of commercial laboratories as a reliable method to diagnose food intolerance. Actually, few data on IgG4 responses to foods in adults are available in the literature. In this study, we evaluated the clinical utility of specific IgG4 dosing against food in adult patients with suspected food allergy/intolerance.

METHODS: A case series of 73 adult patients with suspected food allergy and clinical manifestations of chronic urticaria or other allergy-supposed skin symptoms were tested for specific IgG4 against foods. An open food challenge was carried out for all IgG4-positive food. All positive open tests were controlled by double-blind placebo-controlled food challenge.

RESULTS: Forty-five patients (62%) were IgG4 positive for a number of foods, mainly egg, milk, casein and wheat. None of the patients with IgG4-positive testing showed adverse reactions, neither immediate nor delayed, to the corresponding food.

**CONCLUSION:** In adult patients, testing for specific IgG4 lacks clinical utility for the diagnosis of food allergy or intolerance. Dosing of IgG4 should not be part of the diagnosis and therapy of adult patients with allergy-like skin diseases.

**Czaja-Bulsa G1,2, Bulsa M3, Gębala A4,5. Food IgG4 antibodies are elevated not only in children with wheat allergy but also in children with gastrointestinal diseases. BMC Gastroenterol. 2016 Mar 22;16:39.**

BACKGROUND: Food sIgG and sIgG4 are highly individually versatile. We put a hypothesis that one of the responsible factors is the presence of gastrointestinal inflammatory diseases. The objectives were: 1. An analysis of wheat and rice sIgG and sIgG4 in healthy children, children with IgE-mediated wheat allergy (WA), coeliac disease (CD) and Helicobacter pylori infection (HP). 2. Usability of wheat sIgG and sIgG4 in the WA diagnostics.

METHODS: We compared 388 each wheat and rice sIgG and sIgG4 in a group of 200 children: 50 WA (diagnosis, diet treatment, tolerance), 50 CD (diagnosis and remission), 50 HP and 50 healthy. SIgE, sIgG, sIgG4 were determined with the FEIA method (Pharmacia CAP System).

RESULTS: In healthy children food sIgG were the lowest; no sIgG4 were found. In the CD diagnosis group wheat and rice sIgG and rice sIgG4 were the most common and their concentrations were the highest ($p < .001$, $p < .05$). Wheat sIgG4 were the highest in WA children (diagnosis and tolerance) to fall during the elimination diet ($p < .05$). Wheat and rice sIgG remained the same in all allergy phases. Rice sIgG also did not differ in the class G4.

**CONCLUSION:** 1. Serum concentrations of wheat and rice sIgG and sIgG4 are elevated in children with CD, HP and WA. 2. Sub-clinical incidence of some gastrointestinal inflammatory diseases may be responsible for high individual versatility of food sIgG and sIgG4 concentrations in serum. 3. Wheat sIgG and sIgG4 in children do not correlate with WA clinical picture.

**Hochwaller H, Schulmeister U, Swoboda I, et al. Patients suffering from non-IgE mediated cow's milk intolerance cannot be diagnosed based in IgG subclass or IgA responses to milk allergens. Allergy 2011; 66: 1201-7.**

BACKGROUND: Cow's milk is one of the most common causes of food allergy. In two-thirds of patients, adverse symptoms following milk ingestion are caused by IgE-mediated allergic reactions, whereas for one-third, the mechanisms are unknown. Aim of this study was to investigate whether patients suffering from non-IgE-mediated cow's milk protein intolerance can be distinguished from persons without cow's milk protein intolerance based on serological measurement of IgG and IgA specific for purified cow's milk antigens.

METHODS: We determined IgG(1-4) subclass and IgA antibody levels to purified recombinant αS1-casein, αS2-casein, β-casein, κ-casein, α-lactalbumin, and β-lactoglobulin in four patient groups by ELISA: Patients with IgE-mediated cow's milk allergy (CMA, n=25), patients with non-IgE-mediated cow's milk protein intolerance (CMPI, n=19), patients with gastrointestinal symptoms not associated with cow's milk ingestion (GI, n=15) and control persons without gastrointestinal problems (C, n=26). Cow's milk-specific IgE levels were determined by ImmunoCAP.

RESULTS: Only CMA patients had IgE antibodies to cow's milk. Cow's milk allergic patients mounted the highest IgG(1) and IgG(4) antibody levels to αS1-casein, αS2-casein, β-casein, κ-casein, and α-lactalbumin. No elevated levels of IgG(4) , IgA, and complement-binding IgG subclasses (IgG(1) , IgG(2) , IgG(3) ) to purified cow's milk allergens were found within the CMPI patients compared to persons without cow's milk protein intolerance (GI and C groups).

**CONCLUSION**: Cow's milk protein intolerant patients cannot be distinguished from persons without cow's milk protein intolerance on the basis of IgG subclass or IgA reactivity to cow's milk allergens.

## Position Papers

**Bindslev-Jensen et al. reactions to foods – position paper from the European Academy of Allergology and Clinical Immunology. Allergy 2004;59:690–697.**

**Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel," J. Allergy Clin Immunol 2010; 126:1106-1118 ("Guidelines").**

**Wuthrich B Specific IgG antibodies as markers of adverse reactions to food. Contra! Monogr Allergy 1996;32:226– 227.),**