Scandinavian Journal of Gastroenterology, 2005; 40: 800–807

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Food-specific IgG4 antibody-guided exclusion diet improves symptoms and rectal compliance in irritable bowel syndrome

SAMEER ZAR[1], LYNNE MINCHER[2], MARTIN J. BENSON[3] & DEVINDER KUMAR[4]

[1]*OGEM Department, St George's Hospital Medical School, London, UK,* [2]*Dietetic Department,* [3]*Department of Gastroenterology, St. Helier Hospital, Carshalton, Surrey, UK, and* [4]*Department of Gastrointestinal Surgery, St George's Hospital Medical School, London, UK*

Abstract

Objective. Dietary modification improves symptoms in irritable bowel syndrome (IBS). Identification of offending foods by dietary elimination/re-challenge is cumbersome. IgG4 antibodies to common food antigens are elevated in IBS. The aim of this article was to evaluate the effect of exclusion diet based on IgG4 titres on IBS symptoms and rectal sensitivity and compliance. ***Material and methods***. The study comprised 25 patients with IBS (3 M, 22 F, mean age 43 years, Rome II criteria). IgG4 titres to 16 foods (milk, eggs, cheese, wheat, rice, potatoes, chicken, beef, pork, lamb, soya bean, fish, shrimps, yeast, tomatoes and peanuts) were measured. Foods with titres >250 μg/l were excluded for 6 months. Symptom severity was assessed with a previously validated questionnaire at baseline, at 3 months and at 6 months. Rectal compliance and sensitivity were measured in 12 patients at baseline and at 6 months. ***Results***. IgG4 antibodies to milk, eggs, wheat, beef, pork and lamb were commonly elevated. Significant improvement was reported in pain severity ($p<0.001$), pain frequency ($p=0.034$), bloating severity ($p=0.001$), satisfaction with bowel habits ($p=0.004$) and effect of IBS on life in general ($p=0.008$) at 3 months. Symptom improvement was maintained at 6 months. Rectal compliance was significantly increased ($p=0.011$) at 6 months but the thresholds for urge to defecate/discomfort were unchanged. ***Conclusions***. Food-specific IgG4 antibody-guided exclusion diet improves symptoms in IBS and is associated with an improvement in rectal compliance.

Key Words: *Barostat, exclusion diet, food-specific IgG4 antibody, irritable bowel syndrome, rectal compliance, rectal sensory threshold*

Introduction

The aetiopathogenesis of irritable bowel syndrome (IBS) is thought to be multifactorial involving an interaction between diet [1], infection [2], antibiotics [3] and psychosocial factors [4]. This causes perturbation of the enteric nervous system and sensorimotor dysfunction. Rectal hypersensitivity is a well-recognized characteristic in IBS patients and increased sensory perception and reduced compliance, in response to rectal distension with increasing balloon volume or pressure, have been reported [5,6].

A significant proportion of IBS patients attribute their symptoms to adverse food reactions. Since the initial study by Jones published in 1982, which demonstrated symptomatic response to a 1-week elimination diet, several studies have shown a response rate of up to 67% in IBS patients [1,7,8]. Most studies have used either a standard exclusion diet for all the subjects or excluded foods from the diet based on the history of intolerance as reported by the patients. This approach has been adopted because of a lack of objective testing for food hypersensitivity. Recently, IgG4 antibodies to various food antigens have been incriminated in the pathogenesis of food hypersensitivity-induced atopic conditions [9–11]. A similar finding has been reported in IBS patients, although the exact role of food-specific IgG4 antibodies in the pathogenesis of IBS has not been evaluated [12]. It has been suggested that, similar to other atopic conditions,

Correspondence: Devinder Kumar, PhD, FRCS, Department of Gastrointestinal Surgery, St George's Hospital Medical School, Cranmer Terrace, London SW17 0QT, UK. Tel: +44 020 8725 1302. Fax: +44 020 8725 3611. E-mail: dkumar@sghms.ac.uk

(Received 17 August 2004; accepted 6 December 2004)

ISSN 0036-5521 print/ISSN 1502-7708 online
DOI: 10.1080/00365520510015593

food-specific IgG4 antibodies may be involved in the pathogenesis of a subgroup of IBS patients. Elevated titres to specific food antigens may prove useful for targeted dietary exclusion in a subgroup of IBS patients. This may obviate the need for excluding a large number of foods from the diet which are poorly tolerated and can be nutritionally inadequate. The success of this approach will implicate food-specific IgG4 antibodies in the pathophysiology of IBS.

Abnormalities in sensorimotor physiology of the upper and lower gut have been reported in IBS. The barostat has increasingly been used as a research tool to determine visceral sensitivity thresholds and compliance and the standardization of this technique has been described in detail [13–15]. It has been shown that IBS patients have a lower sensory threshold to volume distension compared to controls, in both the rectum and colon [5,16–18]. Compliance is considered to be an important factor in the reservoir function of the rectum and is reduced in IBS patients [19,20]. These abnormalities in the gut sensorimotor function have been used as markers of response to therapeutic interventions.

The aim of this study was, first, to evaluate the response to food-specific IgG4 antibody-guided exclusion diet on patients' symptoms over a period of 6 months, and secondly, to compare the changes in the rectal visceral sensitivity and compliance as an objective measure of the response to such a diet.

Material and methods

Subjects

Twenty-five IBS patients (3 M, 22 F, mean age 42.6 years, SD ± 14) participated in the dietary exclusion study. The group comprised 13 patients with diarrhoea-predominant symptoms (D-IBS), 10 patients with constipation-predominant symptoms (C-IBS) and 2 with alternating symptoms (Alt-IBS). In a subgroup of 12 patients, rectal compliance and sensory thresholds for the urge to defecate and discomfort were measured using barostat at baseline and repeated after 6 months on the exclusion diet.

The diagnosis of IBS was based on the Rome II criteria [21], defined as abdominal pain/discomfort of at least 12 weeks' duration in the previous one year, which need not be continuous, associated with two of following three criteria; altered stool frequency, altered stool form and/or relief with defecation. A thorough history, physical examination and routine blood tests were carried out in all subjects. Colonoscopy or flexible sigmoidoscopy with barium enema was carried out to exclude bowel pathology. Patients with inflammatory bowel disease, coeliac disease, known lactose intolerance or other significant gastrointestinal disorder were excluded from the study. In addition, "advanced" cardiac, respiratory, renal or hepatic diseases; concurrent malignancy; major psychiatric disorders or a history of drug/alcohol abuse were also considered as exclusion criteria. Patients who had undergone previous abdominal surgery, with the exception of uncomplicated appendicectomy, were also excluded. Use of any medication which could perturb gastrointestinal sensorimotor function was disallowed. Patients who were pregnant or became pregnant during the study were also excluded. The protocol was approved by the local ethics committee and full, written, informed consent was obtained from the subjects.

Symptom severity questionnaire

All patients were asked to complete two questionnaires at baseline. The first questionnaire assessed the symptoms based on the Rome II criteria [21] including the site and frequency of abdominal pain, relief with defecation, stool frequency and stool form. The second questionnaire was used to measure the severity of symptoms using a previously validated questionnaire [22]. Patients were prompted to score their symptoms on a visual analogue scale (range 0–100) for the 10-day period prior to the interview. The first two questions recorded the severity of pain and bloating. Pain was also scored for frequency, thereby giving it a greater influence on the total score. In addition, patients scored the degree of dissatisfaction with their bowel habits and the effect of IBS on their life in general. A composite severity score was then calculated by adding the response to all the above questions. The questionnaire was used to monitor progress in response to the exclusion diet at 3 and 6 months.

Anxiety and depression score

The level of anxiety and depression experienced by the subjects was scored using the Hospital Anxiety and Depression scale (HAD scale) [23]. The questionnaire prompted the subjects to choose the most appropriate response to 14 questions (7 for anxiety and 7 for depression), each scored from 0 to 3 with the higher scores indicating a greater level of anxiety or depression. The questionnaire was repeated again at 3- and 6-month intervals after initiating the exclusion diet.

Food-specific serum IgG4 antibody

Serum IgG4 antibody titres to 16 common foods were measured (milk, eggs, wheat, cheddar cheese, rice, yeast, potato, peanut, fish, chicken, lamb,

beef, pork, tomatoes and soya bean). The samples were processed in a central laboratory (Allergy Diagnostic Laboratory, Oxfordshire, UK) using a commercially available radioimmuno-fluorescence technique (Pharmacia Unicap Autolyser). The technique involves incubating patients' serum with a cellulose cap containing the food antigen of interest. After washing away the excess antibodies, the cap is incubated with fluorescence-labelled mouse anti-IgG antibody, which is then measured using a fluorometer after enzyme activation. The measured range of antibodies is between 1.5 mcg/l and 30,000 mcg/l. A cut-off value of 250 mcg/l was used to exclude the foods tested.

Exclusion diet

This was done under the supervision of a qualified dietician. Patients had a detailed interview with the dietician where a complete dietary history was obtained. In addition, they were also asked to maintain diary cards with details of their dietary intake for a 2-week period prior to the initial interview. This information helped in assessing how much of the suspected foods were being consumed by the patient so that appropriate alternatives could be suggested to replenish the intake once the patient started the exclusion diet. This was especially important for the patients who were excluding several types of foods, in order to ensure they continued to eat a nutritionally balanced diet. The results of the IgG4 antibody tests were then discussed with the patients and the foods that induced antibody titres greater than 250 mcg/l were excluded from the diet. The patients were also advised to check the contents of the pre-prepared foods and a list of the suspected foods was supplied for their reference. Patients were reviewed at one month from the start of the exclusion diet to assess their progress, reinforce the dietary advice and answer any queries they might have. Patients were reviewed at 3 and 6 month when their symptoms were assessed as described above while reinforcing the dietary advice.

Barostat and catheter assembly

A barostat (Synectics Medical, Copenhagen, Denmark) coupled with a disposable, 500 ml polyethylene bag secured to the distal 5 cm of a multilumen polyvinyl catheter was used to measure rectal compliance and the pressure thresholds for the urge to defecate and pain/discomfort. This was carried out using a phasic pressure sensitivity procedure in a double random staircase sequence. Incremental pressure steps (multiples of 4 mmHg) of 20-s duration separated by a 30-s rest period were used. The thresholds for urge to defecate and discomfort/pain were determined using this technique. A pressure-volume relationship was determined for each patient by plotting the corresponding pressures and volumes for each inflation step and rectal compliance (ml/mmHg) was calculated from the slope of the pressure-volume curve using simple regression.

Data and statistical analysis

The baseline symptom severity scores (range 0–100) obtained on the visual analogue scales were compared with the scores at 3 and 6 months. Similarly, the baseline rectal compliance and sensory thresholds for urge to defecate and discomfort were compared with the data obtained on repeat testing at 6 months. A paired *t*-test was used for statistical analysis using SPSS version 11.

Results

IgG4 antibody titres and the exclusion diet

The food articles against which IgG4 antibody titres were most commonly elevated (>250 μg/l) were milk, cheddar cheese, eggs, beef, pork, lamb, wheat and tomatoes (Table I). These were elevated in more than 50% of patients. Antibody titres to chicken, fish, rice, yeast, potatoes, soya bean, peanuts and shrimps were elevated in fewer patients and for these foods the observed titres were <500 μg/l in most instances.

On average each patient excluded 8 (range 3–13) foods from the diet based on the IgG4 titres >250 μg/l. The dietary advice was reinforced by the dietician at the 1-month, 3-month and 6-month interviews. The patients reported full compliance to the exclusion diet for the duration of the study. The results of the blood tests were shown to the subjects, which helped in motivating them. In addition, advice from the dietician ensured that patients were able to choose alternatives to the excluded foods, thereby further facilitating compliance.

Symptom scores

The baseline symptom profile was compared with the response at 3 and 6 months. The symptoms were scored between 0 and 100 using a visual analogue scale, where 0 signified no adverse symptoms. Data were available from 21/25 patients at 3 months and showed significant improvement as compared to baseline in pain severity (0 IQR$\pm$39 versus 61 IQR$\pm$39, $p<0.001$), pain frequency (10 IQR$\pm$45 versus 50 IQR$\pm$50, $p=0.034$), bloating severity (27 IQR$\pm$50 versus 51 IQR$\pm$24,

Table I. Food specific serum IgG4 titres in IBS patients.

Food antigen tested	Titres >250 mcg/l	Titres >500 mcg/l	Titres >1000 mcg/l
Milk	25/25	25/25	25/25
Cheese	19/25	19/25	15/25
Eggs	23/25	20/25	16/25
Beef	24/25	23/25	14/25
Pork	21/25	15/25	3/25
Lamb	13/25	5/25	1/25
Fish	0/25	0/25	0/25
Chicken	2/25	1/25	0/25
Shrimps	1/25	0/25	0/25
Wheat	16/25	12/25	9/25
Rice	2/25	0/25	0/25
Potatoes	3/25	2/25	1/25
Peanuts	7/25	2/25	1/25
Tomatoes	13/25	9/25	4/25
Soya	5/25	3/25	1/25
Yeast	2/25	0/25	0/25

Abbreviation: IBS = irritable bowel syndrome.

$p = 0.001$), satisfaction with bowel habits (35 IQR$\pm$70 versus 70 IQR$\pm$24, $p = 0.004$) and effect of IBS on life in general (34 IQR$\pm$49 versus 70 IQR$\pm$27, $p = 0.008$) (Table II, Figure 1). The total score was also significantly improved (123 IQR$\pm$215 versus 309 IQR$\pm$104, $p < 0.001$) at 3 months.

Symptom severity score data at 6 months were available for 15 patients. Of the remaining 10 patients, 1 discontinued the diet because of family problems although having a good initial response, 1 patient was diagnosed with a gynaecological condition requiring surgery, 1 patient moved away from the area and 1 was working in the entertainment industry and found the dietary restrictions incompatible with lifestyle. The remaining 6 patients were lost to follow-up. For the patients who completed the study, the results of the 6-month data were comparable with the 3-month data showing that improvement in all the parameters had been maintained. A significant improvement in pain severity (35 IQR$\pm$43, $p = 0.005$), pain frequency (50 IQR$\pm$60, $p = 0.027$), bloating severity (50 IQR$\pm$42, $p = 0.024$), satisfaction with bowel habits (66 IQR$\pm$58, $p = 0.016$) and effect of IBS on life in general (51 IQR$\pm$55, $p = 0.001$) was observed (Table II, Figure 1). The total score was also significantly improved (207 IQR$\pm$220, $p = 0.002$) at 6 months.

Anxiety and depression scores

The baseline anxiety and depression scores calculated from the HAD questionnaire were not significantly changed at 3 months. However, at 6 months, a significant improvement in the mean scores for

Table II. Effect of exclusion diet on symptom severity scores.

	Baseline Median$\pm$IQR	3 months Median$\pm$IQR p-value[†]	6 months Median$\pm$IQR p-value[†]
Pain severity	61$\pm$36	0$\pm$39 $p < 0.001$	35$\pm$43 $p = 0.005$
Pain frequency	50$\pm$50	10$\pm$45 $p = 0.034$	50$\pm$60 $p = 0.027$
Bloating severity	51$\pm$24	27$\pm$50 $p = 0.001$	50$\pm$42 $p = 0.024$
Bowel habits	70$\pm$24	35$\pm$70 $p = 0.004$	66$\pm$58 $p = 0.016$
Effect on life in general	70$\pm$27	34$\pm$49 $p = 0.008$	51$\pm$55 $p = 0.001$
Total scores	309$\pm$104	123$\pm$215 $p < 0.001$	207$\pm$220 $p = 0.002$

Values are represented as the median and interquartile range (IQR).
[†]p-values are versus baseline scores.

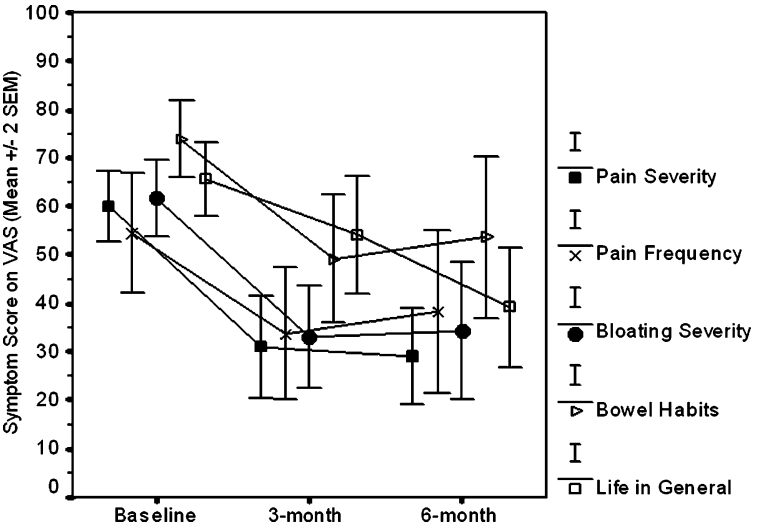

Figure 1. At 3 months and 6 months, there was significant improvement in pain severity ($p < 0.001$, $p = 0.005$), pain frequency ($p = 0.034$, $p = 0.027$), bloating severity ($p = 0.001$, $p = 0.024$), satisfaction with bowel habits ($p = 0.004$, $p = 0.016$) and the effect of irritable bowel syndrome (IBS) on life in general ($p = 0.008$, $p = 0.002$) versus baseline.

both anxiety and depression was seen compared to the baseline scores (4 IQR$\pm$4 versus 2 IQR$\pm$4, $p = 0.007$ for depression score; 10 IQR$\pm$5 versus 7 IQR$\pm$5, $p = 0.015$ for the anxiety score) (Table III).

Rectal compliance and hypersensitivity

Rectal compliance and the thresholds for discomfort and the urge to defecate were measured using a barostat at baseline and after 6 months on the exclusion diet. A significant increase in rectal compliance was seen at 6 months as compared to baseline in response to the exclusion diet (5.42 ml/mmHg SD$\pm$2.33 baseline versus 7.75 ml/mmHg SD$\pm$4.22 at 6 months; $p = 0.011$) (Figure 2). No significant change was observed in the pressure thresholds for discomfort/pain or the urge to defecate after 6 months of being on the exclusion diet (Table IV).

Discussion

The management of IBS has traditionally been based on reassurance and symptomatic treatment. Evidence from dietary elimination and re-challenge studies supports the role of diet in IBS. This study demonstrates that IBS patients experience a significant improvement in symptoms in response to a "food-specific IgG4"-guided exclusion diet. The diet was based on elevated IgG4 antibody titres to 16 common articles of food. The most common foods associated with elevated IgG4 titres were beef, pork, wheat and dairy products. This subjective symptomatic improvement reported by the patients was mirrored by objective evidence of physiological change in the sensorimotor function (i.e. increase in rectal compliance).

Symptom severity was assessed by a previously validated questionnaire in which the patients ranked bloating and pain severity, pain frequency, satisfaction with bowel habits and effect/interference of IBS on life in general on a visual analogue scale. At 3 months, a significant improvement was seen in IBS symptoms, i.e. pain severity, pain frequency, bloating severity and satisfaction with bowel habits. In addition, the IBS symptoms interfered significantly less with their lives in general since the introduction of the exclusion diet. The composite scores were also significantly improved confirming that improvement in one parameter is not offset by deterioration in another. The improvement observed at 3 months was maintained at the 6-month follow-up for all the

Table III. Effect of exclusion diet on hospital anxiety and depression scores.

	Baseline Median$\pm$IQR	3 months Median$\pm$IQR	6 months Median$\pm$IQR
Anxiety score	10$\pm$5	8$\pm$5	7$\pm$5†
Depression score	4$\pm$4	2$\pm$4	1$\pm$3‡

Values are represented as the median and interquartile range (IQR).
$^{\dagger}p = 0.018$ (baseline versus 6 months); $^{\ddagger}p = 0.002$ (baseline versus 6 months).

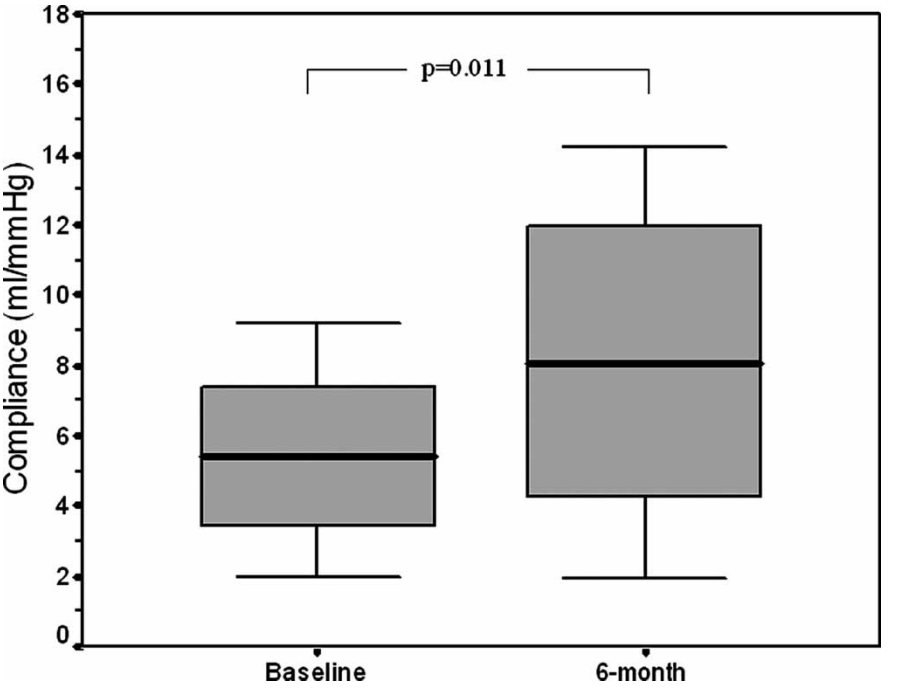

Figure 2. The exclusion diet resulted in an increase in rectal compliance (ml/mmHg) at 6 months versus baseline (7.75 versus 5.42, $p = 0.011$).

parameters and patients continued to benefit from the exclusion diet. Specific data on the effect of the exclusion diet on bowel frequency was not collected and would have been difficult to interpret, as the number of subjects in each IBS subgroup was small. However, the overall improvement in bowel habits reported by the subjects suggests that the intervention may have had a positive effect on bowel frequency. However, larger studies in the future will be needed specifically to answer this question.

Previous exclusion diet studies have shown a similar response rate to exclusion diets. It is technically difficult to perform double-blind exclusion diet studies. Most of the studies have used open dietary elimination and challenge design and therefore placebo response cannot be excluded. Blinding the patient would require changing the appearance of the food, e.g. liquidized form and administering food through nasogastric tubes to blind the patient to the taste and smell of the food. Organizing studies where patients can be blinded to the food in such a manner over a prolonged period of time is impractical. Unfortunately, this means that placebo response cannot be excluded with certainty. However, the persistence of improvement in symptoms at 6 months and the fact that it was associated with objective physiological improvement in rectal compliance suggests that the response is likely to be real.

Currently, two empirical methods are used in clinical practice for prescribing exclusion diets. The first method involves a hypoallergenic diet consisting of a small number of "allowed" foods. Once the symptoms are controlled, excluded foods are reintroduced one by one while monitoring for symptom recurrence. This approach is very restrictive and cumbersome, although it is likely to benefit patients who have hypersensitivity to a large number of foods. The second approach is to exclude foods based on history and/or to exclude a small number of foods commonly associated with the disorder and then

Table IV. Effect of exclusion diet on rectal sensory thresholds and compliance.

	Compliance (ml/mmHg)[†]		Discomfort (mmHg)		Mean urge to defecate (mmHg)	
	Mean	SD	Mean	SD	Mean	SD
Baseline	5.42	2.33	28.42	11.20	19.16	8.12
6 months	7.75	4.22	23.72	5.25	21.68	7.61

[†]$p = 0.011$.

exclude additional foods if the symptoms persist. This method may take a long time to be effective, especially if the "offending" foods are atypical. These techniques are employed primarily because there is no objective way of testing food hypersensitivity available at present.

Unlike the two approaches discussed above, the exclusion diets employed in this study were tailored to the individual patient based on the serum IgG4 antibody titres. This "targeted" approach has many advantages. First, it provides objectivity to the process by excluding only those articles of food that are associated with increased antibody titres. Patients are more likely to be compliant as it is more "scientific". Secondly, the diet is individualized to a given patient thereby obviating the need for excluding a large number of foods from the diet. Thirdly, this approach is likely to reduce the number of consultations needed. Lastly, the clinicians are likely to be more confident in prescribing exclusion diets based on objective testing.

This study also raises many questions. It should be pointed out that antibody titres to only 16 common foods were tested and this panel may not be enough for all the patients. In addition, an arbitrary cut-off level of 250 μg/l was used. Whether the precise cut-off level can be increased or reduced is not known and whether different cut-off levels should be used for different articles of food remains to be determined. It is also not known if this approach would lead to reduction in the antibody titres, which may be useful for monitoring progress. Further studies are needed to determine whether the excluded foods could be reintroduced into the diet once the symptoms have been resolved, and, if so, in what quantity.

Previous exclusion diet studies have used symptom assessment alone in determining the efficacy of dietary interventions in IBS patients. This is the first study which demonstrates that symptom improvement is associated with changes in objective physiological data, i.e. an increase in rectal compliance as measured with a barostat. Demonstration of a change in the gut sensorimotor function in response to the exclusion diet supports a cause and effect relationship. However, the exclusion diet did not show any significant change in the sensory thresholds for urge to defecate and discomfort. This may partly be due to the fact that the study population included a mixture of diarrhoea, constipation and alternating symptom IBS patients and these may have different response patterns to sensory testing. In addition, once established, mucosal hypersensitivity might be irreversible or take a longer time to revert to normality and repeat testing at a longer duration of follow-up might have shown significant results.

It was also noted that anxiety and depression scores improved significantly at 6 months but not at 3 months. This delayed improvement suggests that the heightened level of anxiety and depression experienced by IBS patients may be secondary to the impact of the symptoms on their life rather than a primary trigger. Once the symptoms resolve, the levels of anxiety and depression return to baseline.

In summary, exclusion diet based on food-specific serum IgG4 antibody testing may provide a useful adjunct in IBS patients with persistent symptoms or those with a clear history of adverse food reactions. Serum IgG4 antibody testing may provide an objective and quick method for selecting an exclusion diet for treating such patients.

References

[1] Jones VA, McLaughlan P, Shorthouse M, Workman E, Hunter JO. Food intolerance: a major factor in the pathogenesis of irritable bowel syndrome. Lancet 1982;2(8308): 1115–7.

[2] McKendrick MW, Read NW. Irritable bowel syndrome: post salmonella infection [see comments]. J Infect 1994;29:1–3.

[3] Mendall MA, Kumar D. Antibiotic use, childhood affluence and irritable bowel syndrome (IBS). Eur J Gastroenterol Hepatol 1998;10:59–62.

[4] Drossman DA, McKee DC, Sandler RS, Mitchell CM, Cramer EM, Lowman BC, et al. Psychosocial factors in the irritable bowel syndrome. A multivariate study of patients and nonpatients with irritable bowel syndrome. Gastroenterology 1988;95:701–8.

[5] Mertz H, Naliboff B, Munakata J, Niazi N, Mayer EA. Altered rectal perception is a biological marker of patients with irritable bowel syndrome [published erratum appears in Gastroenterology 1997;113:1054]. Gastroenterology 1995; 109:40–52.

[6] Distrutti E, Salvioli B, Azpiroz F, Malagelada JR. Rectal function and bowel habit in irritable bowel syndrome. Am J Gastroenterology 2004;99:131–7.

[7] Bentley SJ, Pearson DJ, Rix KJ. Food hypersensitivity in irritable bowel syndrome. Lancet 1983;2(8345):295–7.

[8] Petitpierre M, Gumowski P, Girard JP. Irritable bowel syndrome and hypersensitivity to food. Ann Allergy 1985;54: 538–40.

[9] Awazuhara H, Kawai H, Maruchi N. Major allergens in soybean and clinical significance of IgG4 antibodies investigated by IgE- and IgG4-immunoblotting with sera from soybean-sensitive patients. Clin Exp Allergy 1997;27:325–32.

[10] el Rafei A, Peters SM, Harris N, Bellanti JA. Diagnostic value of IgG4 measurements in patients with food allergy. Ann Allergy 1989;62:94–9.

[11] Nakagawa T, Okano Y, Iwasaki E, Akimoto K, Nagakura T, Iikura Y, et al. Clinical significance of IgG4 antibody determination in children against egg white, milk, soybean and *Dermatophagoides farinae*. Arerugi 1992;41:1694–704.

[12] Zar S, Benson MJ, Kumar D. Serum IgG4 antibodies to common food antigens are elevated in irritable bowel syndrome [abstract no. 092]. Gut 2002;50 Suppl 11:A25.

[13] Whitehead WE, Delvaux M. Standardization of barostat procedures for testing smooth muscle tone and sensory thresholds in the gastrointestinal tract. The Working Team of Glaxo-Wellcome Research, UK [see comments]. Dig Dis Sci 1997;42:223–41.

[14] van der Schaar PJ, Lamers CB, Masclee AA. The role of the barostat in human research and clinical practice [In Process Citation]. Scand J Gastroenterol Suppl 1999;230:52–63.

[15] Azpiroz F, Malagelada JR. Physiological variations in canine gastric tone measured by an electronic barostat. Am J Physiol 1985;248(2 Pt 1):G229–37.

[16] Bradette M, Delvaux M, Staumont G, Fioramonti J, Bueno L, Frexinos J. Evaluation of colonic sensory thresholds in IBS patients using a barostat. Definition of optimal conditions and comparison with healthy subjects. Dig Dis Sci 1994;39:449–57.

[17] Whitehead WE, Holtkotter B, Enck P, Hoelzl R, Holmes KD, Anthony J, et al. Tolerance for rectosigmoid distension in irritable bowel syndrome. Gastroenterology 1990;98: 1187–92.

[18] Hammer J, Phillips SF, Talley NJ, Camilleri M. Effect of a 5HT3-antagonist (ondansetron) on rectal sensitivity and compliance in health and the irritable bowel syndrome. Aliment Pharmacol Ther 1993;7:543–51.

[19] Salvioli B, Bharucha AE, Rath-Harvey D, Pemberton JH, Phillips SF. Rectal compliance, capacity, and rectoanal sensation in fecal incontinence. Am J Gastroenterol 2001;96:2158–68.

[20] Kwan CL, Davis KD, Mikula K, Diamant NE. Abnormal rectal motor physiology in patients with irritable bowel syndrome. Neurogastroenterol Motil 2004;16:251–63.

[21] Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Muller-Lissner SA. Functional bowel disorders and functional abdominal pain. Gut 1999;45 Suppl II:II43–7.

[22] Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther 1997;11:395–402.

[23] Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;67:361–70.