Dose dependent effects of protracted ingestion of small amounts of gliadin in coeliac disease children: a clinical and jejunal morphometric study

C Catassi, M Rossini, I-M Rätsch, I Bearzi, A Santinelli, R Castagnani, E Pisani, G V Coppa, P L Giorgi

Abstract

This study aimed to investigate the effects of chronic ingestion of small amounts of gliadin on children with coeliac disease. A four week challenge was performed on 20 children who had been on a gluten free diet for mean (SD) 14 (3) months. They were given a daily dose of either 100 mg (group A, n=10, mean age 4 (2) years) or 500 mg of gliadin (group B, mean age 5 (3) years). The effects of the gliadin were monitored by morphometric study of the jejunal mucosa, intestinal permeability test with cellobiose/mannitol, and serum anti-gliadin antibody test. After the challenge, group A patients showed a significant increase in the mean intraepithelial lymphocyte count (before challenge 11 (3), afterwards 19 (6)) and a decrease in the villous height/crypt depth ratio (beforehand 1·5 (0·1), afterwards 1·3 (0·2)), while the intestinal permeability test remained normal and the IgA-antigliadin antibody increased in four of 10 children. After the challenge group B showed more pronounced histological changes, an increase in the mean urinary cellobiose/mannitol % (beforehand 0·028 (0·020), afterwards 0·058 (0·028)), and IgA-antigliadin antibody positivity in six of eight subjects. The discriminant analysis function showed that the pretreatment group, group A after challenge, and group B after challenge were correctly classified in 90% of cases by functions based on the individual intraepithelial lymphocyte count and the villous height/crypt depth ratio. This study shows that chronic ingestion of small amounts of gluten causes dose-dependent damage to the small intestinal mucosa in children with coeliac disease. The predictive value of laboratory tests, such as the antigliadin antibody test and the intestinal permeability test seems to be lower in treated patients than in those with active coeliac disease.

(*Gut* 1993; **34**: 1515–1519)

Department of Paediatrics, University of Ancona, Ancona, Italy
C Catassi
M Rossini
I M Rätsch
G V Coppa
P L Giorgi

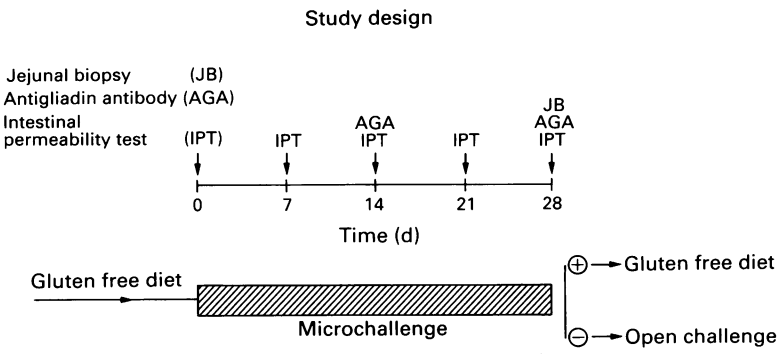
Department of Pathology, University of Ancona, Italy
I Bearzi
A Santinelli

CEDUA, University of Ancona, Italy
R Castagnani
E Pisani

Correspondence to:
Dr C Catassi, Clinica Pediatrica Università, via Corridoni 11, 60123 Ancona, Italy.

Accepted for publication 10 March 1993

Figure 1: Timing of the gliadin microchallenge.



Although the causal role of gluten in coeliac disease is well established, the relationship between the quantity of gluten ingested and the severity of the histological and clinical abnormalities in coeliac disease patients is still unclear, especially for small amounts of gluten. This issue could have practical relevance in the follow up of coeliac disease patients who may seem healthy during dietary treatment but still suffer from some degree of jejunal mucosa damage because of the ingestion of 'hidden' gluten.[1]

Several studies have shown the consequences of a gluten challenge in coeliac disease subjects, whether as a single amount[2,3] or as repeated daily administration of gluten within the range of a normal diet.[4,5] However, information on the effects of chronic ingestion of small doses of gluten (or gliadin) in coeliac disease patients is rather scarce. We therefore studied 20 coeliac disease children who underwent a four week challenge with either 100 or 500 mg of gliadin per day. We report here the results of morphometric study of the jejunal mucosa and of laboratory evaluation (the antigliadin antibody test and sugar intestinal permeability test) in these subjects.

Patients and methods

PATIENTS

We studied 20 children with coeliac disease who had been following a gluten free diet for mean (SD) 14 (3) months. They were admitted to our department between January 1990 and December 1991. Diagnosis of coeliac disease was based on subtotal villous atrophy in the first biopsy specimen and subsequent improvement of morphology in a repeat jejunal biopsy specimen taken after the gluten free diet. The gliadin challenge described here represented the first phase of the diagnostic gluten challenge adopted by the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN).[6] Two weeks before joining the study the degree of compliance with dietary treatment was assessed by a dietitian and a standard gluten free diet, including commercially available gluten free products, was recommended. Available information[7] suggests that the gliadin content of the standard gluten free diet is about 1 mg per day. The patients' parents gave their informed consent to the study. These 20 patients were taken from a total of 32 diagnosed during the same period; 12 patients were excluded because of poor compliance with the diet (seven cases) or denied consent to the protocol (five cases).

TABLE I *Individual results of the gliadin microchallenge in the patients studied*

		T_0					T_1				
	Age (y)/ sex	VH (μm)	CD (μm)	VH/CD	IEL (×100)	C/M %	VH (μm)	CD (μm)	VH/CD	IEL (×100)	C/M %
Group A:											
1	4·6/F	218	153	1·42	10	0·032	231	200	1·15	13	0·007
2	2·9/F	340	204	1·67	14	0·037	211	197	1·07	22	0·047
3	1·9/F	237	169	1·4	5	0·057	302	247	1·22	9	0·037
4	3·1/F	218	127	1·72	6	0·032	249	164	1·51	18	0·047
5	1·7/M	288	187	1·54	13	0·022	239	187	1·28	23	0·062
6	5·6/F	286	178	1·61	12	0·041	250	161	1·55	31	0·017
7	2·7/F	293	179	1·63	12	0·041	318	218	1·46	19	0·069
8	2·1/M	304	236	1·29	9	0	277	189	1·47	14	0
9	9·1/F	199	135	1·47	12	0·011	297	242	1·23	19	0·019
10	6·1/F	308	214	1·44	14	0·01	254	198	1·28	20	0·023
Group B:											
11	3·1/M	233	153	1·52	9	0·021	206	170	1·21	28	0·099
12	8·1/F	280	163	1·72	11	0·024	240	225	1·07	29	0·08
13	2·6/M	261	190	1·37	11	0·018	209	172	1·21	18	0·037
14	1·6/M	244	150	1·62	10	0	192	167	1·15	17	0·019
15	6·3/M	288	162	1·78	9	0·056	165	158	1·04	21	0·042
16	3·0/F	238	143	1·66	11	0·009	216	184	1·17	31	0·079
17	3·3/M	217	145	1·5	9	0·063	246	220	1·11	30	0·094
18	6·1/F	251	158	1·59	10	0·03	210	185	1·14	25	0·05
19	9·6/F	282	178	1·58	11	0·02	241	206	1·17	29	0·028
20	2·6/M	221	138	1·6	9	0·037	180	165	1·09	21	0·051

T_0=evaluation before challenge; T_1=evaluation at the end of the four week microchallenge.
VH=villous height; CD=crypt depth; IEL=intraepithelial lymphocyte count;
C/M %=cellobiose/mannitol %.

STUDY DESIGN

The study design is shown in Figure 1. After the basal evaluation (T_0), patients were randomly assigned to either group A or B. Group A comprised 10 children (two boys and eight girls with a mean age of 4·0 (2·4) years) and group B comprised 10 subjects (six boys and four girls with a mean age of 4·7 (2·7) years). Two coeliac disease patients in group B showed a selective IgA deficiency.

During the four week gluten microchallenge the standard gluten free diet was supplemented with 100 mg/day of gliadin (crude gliadin, Sigma Chemical Company, St Louis, MO, USA) in group A children and 500 mg/day of gliadin in group B. The daily gliadin was administered with some sugar in a single dose, 15 minutes before breakfast. Patients were seen at the department after two and four weeks (T_1) to assess dietary compliance and for clinical and laboratory monitoring. The timing of the jejunal biopsies, antigliadin antibody test, and sugar intestinal permeability test is shown in Figure 1. The one week and three week intestinal permeability tests were performed by patients at home. Patients who did not show evidence of relapse after this four week microchallenge had the diagnosis of coeliac disease confirmed by an open challenge with higher doses of gluten.

METHODS

Peroral jejunal biopsy specimens were obtained by a Watson capsule from the area of the ligament of Traitz under fluoroscopic control. Specimens were fixed in 10% formalin, embedded in paraffin wax, sectioned at 5 μm thickness and stained with haematoxylin and eosin. Only well-oriented sections were examined. The morphometric analysis of the sections was performed on at least 10 villi by a computerised image analyser IBAS-AT Kontron (Munich, Germany). The following morphometric parameters were evaluated: villous height, crypt depth, villous height/crypt depth ratio, and the intraepithelial lymphocyte count. The number of intraepithelial lymphocytes for a total of 1000 enterocytes was counted and expressed as intraepithelial lymphocytes per 100 enterocytes, as previously described.[8]

Antigliadin antibodies of IgG and IgA classes were measured in serum samples by an ELISA technique.[9] For the sugar intestinal permeability test, an isotonic acqueous solution of cellobiose (5 g) and mannitol (2 g) was given orally after an overnight fast. All the urine passed during the following five hours was collected and the volume was measured. An aliquot was stored at −20°C. Urinary sugars were determined by a previously described HPLC method.[10] The result of the intestinal permeability test was expressed as the ratio between the percentage of cellobiose and the percentage of mannitol recovered from the urine (cellobiose/mannitol %).

STATISTICAL ANALYSIS

Data are presented as mean (SD). Differences between group means were evaluated by non-parametric tests (the Mann-Whitney test for non-paired data and the Wilcoxon matched pairs test). The relationships between variables were evaluated by the simple correlation coefficient and a multiple regression analysis. The morphometric parameters of the jejunal biopsy specimens before and after challenge were analysed by the discriminant function analysis. All the statistical tests were performed by a computer using SPSS-X software.

Results

All patients completed the study protocol. At the end of the four week period, no clinical abnormality was found in group A patients while signs of active disease (anorexia, pale stools) were reported in three subjects from group B (patient nos 12, 16, and 20), who showed evidence of laboratory and histological relapse at the T_1 evaluation. The persistence of the gluten sensitive enteropathy was definitely assessed in the other 17 patients by an open challenge with normal amounts of daily gluten.

The individual demographic and clinical data are presented in Table I. No significant difference was found in the mean values of the prechallenge morphometric parameters between groups A and B, that is the two groups were histologically homogeneous in the basal condition. The results of the prechallenge and post-challenge morphometric studies and laboratory investigations are shown in Table II and Figures

TABLE II *Results of the jejunal morphometric study and of the sugar intestinal permeability test (mean (SD)) in the study groups*

	Gliadin 100 mg/day			*Gliadin 500 mg/day*		
	T_0		T_1	T_0		T_1
Jejunal morphometry:						
Villous height (μm)	269 (47)		263 (34)	251 (26)	*	210 (27)†
Crypt depth (μm)	178 (34)		200 (29)	158 (16)	*	185 (24)
Villous height/crypt depth	1·5 (0·1)	*	1·3 (0·2)	1·6 (0·1)	*	1·1 (0·1)†
Intraepithelial lymphocytes (×100)	11 (3)	*	19 (6)	10 (1)	*	25 (5)
Intestinal permeability test:						
Cellobiose/mannitol % (×100)	2·8 (1·7)		3·3 (2·3)	2·8 (2·0)		5·8 (2·8)

*T_1 *v* T_0: $p<0·01$; †T_1-500 mg *v* T_1-100 mg: $p<0·01$.

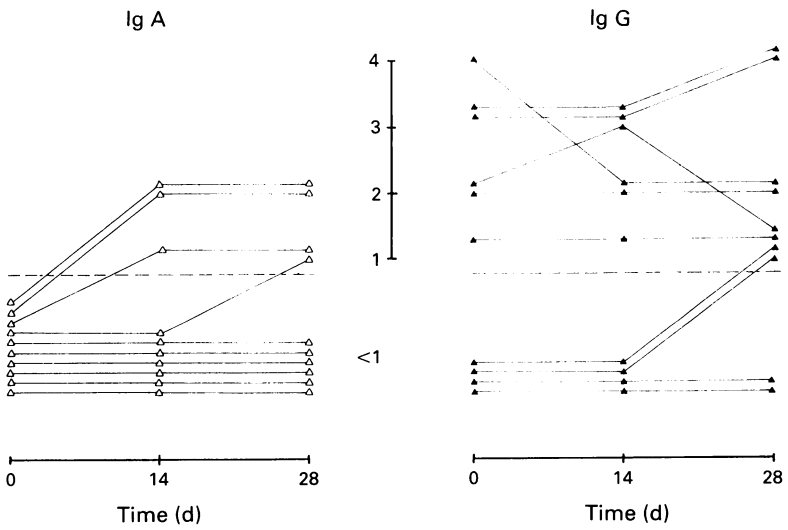


Figure 2: Results of the antigliadin antibody test in group A patients (given 100 mg/d gliadin). The normal result is below the dotted line.

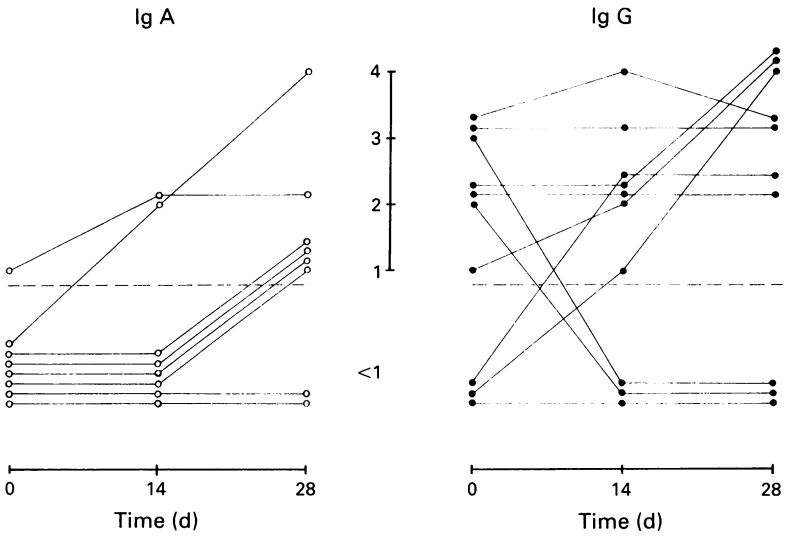


Figure 3: Results of the antigliadin antibody test in group B patients (given 500 mg/d gliadin). The normal result is below the dotted line.

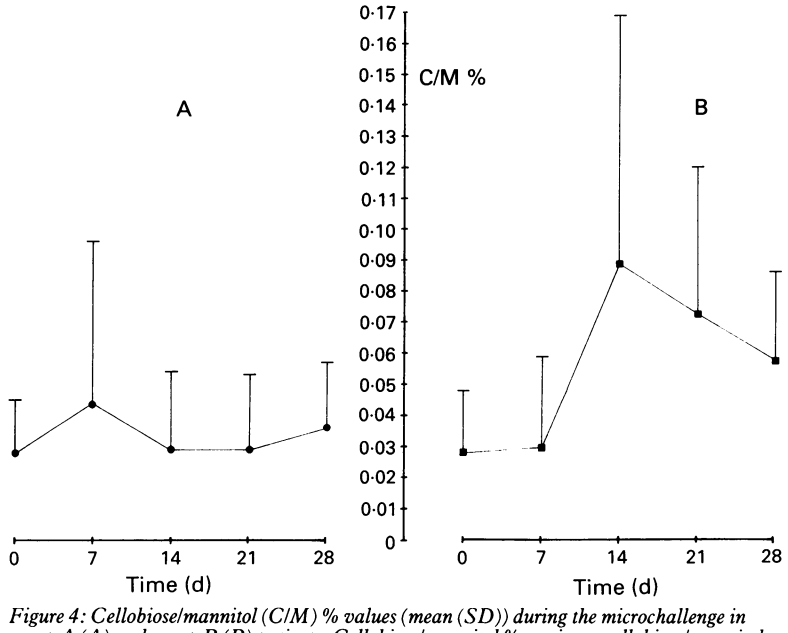


Figure 4: Cellobiose/mannitol (C/M) % values (mean (SD)) during the microchallenge in group A (A) and group B (B) patients. Cellobiose/mannitol % = urinary cellobiose/mannitol recovery %.

2–4. At time T_0 the intraepithelial lymphocyte count was normal in all patients (normal values 8 (3)) but it showed a clear cut increase at time T_1 in both group A and group B patients. No other morphometric or laboratory parameter showed such a constant trend at the individual level.

Before the microchallenge, five of 20 subjects (three in group A and two in group B) showed a slighty abnormal cellobiose/mannitol % value (upper normal limit: 0·04). After the microchallenge an abnormal value was found in four of 10 children from group A and seven of 10 children from group B. The intestinal permeability test result in the whole study group, expressed as cellobiose/mannitol %, was significantly correlated with the villous height/crypt depth ratio ($r=-0·323$; $p=0·042$) and with the intraepithelial lymphocyte count ($r=0·50$; $p=0·01$), but not with villous height or crypt depth. Multiple regression analysis showed no increase in the correlation when the villous height/crypt depth ratio and intraepithelial lymphocyte count together were compared with the cellobiose/mannitol % (multiple $r=0·50$).

To show whether simultaneous consideration of different morphometric measurements could discriminate between differently treated groups, the data were analysed by discriminant analysis. A discussion of the use of this method in the morphometric analysis of jejunal mucosa specimens is given by Penna *et al.*[11] Analysis of the 40 children's biopsy specimens showed that the intraepithelial lymphocyte count and the villous height/crypt depth ratio values allowed separation into groups (with minimal overlap) corresponding to (a) the prechallenge state (group A and B together), (b) postchallenge with 100 mg/day of gliadin, and (c) postchallenge with 500 mg/day of gliadin. For the first canonical discriminant function the standardised coefficients were −0·76732 for villous height/crypt depth ratio and 0·78201 for intraepithelial lymphocyte; for the second canonical discriminant function the standardised coefficients were 0·66324 for villous height/crypt depth ratio and 0·64585 for intraepithelial lymphocyte count. The unstandardised canonical discriminant function coefficients were (a) function 1: −6·069984 (villous height/crypt depth ratio), 0·1825418 (intraepithelial lymphocyte), 5·515047 (constant); (b) function 2: 5·246663 (villous height/crypt depth ratio), 0·1507579 (intraepithelial lymphocyte), −9·734492 (constant). Figure 5 shows that the resolution of the two canonical discriminant functions separates the T_0 groups A and B (left) from the T_1 group A (centre) and the T_1 group B (right), with only four cases out of 40 being misclassified. The predicted group membership corresponded to the actual group in 100% of cases in the basal condition, 80% in the 100 mg/day gliadin microchallenge group (20% were misclassified in the other groups), 80% in the 500 mg/day gliadin microchallenge (20% were misclassified in the 100 mg/day group).

Discussion

The major finding of this study is that the effects of chronic ingestion of a small amount of gliadin

in this group of coeliac disease children were clearly dose-dependent. After a four week microchallenge with 100 mg/day of gliadin (roughly equivalent to 200 mg of gluten or to 2·5 g of wheat flour) there were minimal morphometric changes in the jejunal histology. Jejunal mucosal changes were more pronounced in the patients who had received 500 mg/day gliadin. In patients taking 500 mg/day gliadin, the laboratory investigations often yielded abnormal results and clinical signs of relapse were also seen in some. The dose-effect relationship was confirmed by discriminant function analysis of the morphometric variables, which showed that the pretreatment group, the 100 mg/day challenge group, and the 500 mg/day challenge group were correctly classified in 90% of cases by functions including the individual intraepithelial lymphocyte count and villous height/crypt depth ratio (Fig 5).

It is interesting that the dose-response effect we found in these coeliac disease children is similar to that found in the single gluten challenge study by Ciclitira *et al*, which showed no evidence of jejunal mucosal damage after the infusion of 10 mg of gliadin, minimal changes after 100 mg of gliadin, and noticeable histological changes after an additional 500 mg challenge.[12] With single doses of 0·1 to 1·5 g of gluten using Frazer's fraction III, Leigh *et al* found a time dependent dose related increase in intraepithelial lymphocyte after 12 hours, but no changes in mucosal architecture.[2] Taken as a whole, these data could indicate that a 100 mg dose of gliadin is near the threshold for morphologically detectable intestinal damage in most coeliac disease patients. This is not to say that lower gliadin doses are not potentially harmful to coeliac disease subjects. Previous studies have reported that the chronic ingestion of less than 10 mg of gliadin daily is occasionally sufficient to cause problems in coeliac disease patients.[7 13-15] It has also been shown that an acute challenge with 12–15 mg of gliadin induces an increased intestinal secretion of prostaglandin E_2,[16] albumin, hyaluronan, and beta$_2$-microglobulin[17] in coeliac disease subjects.

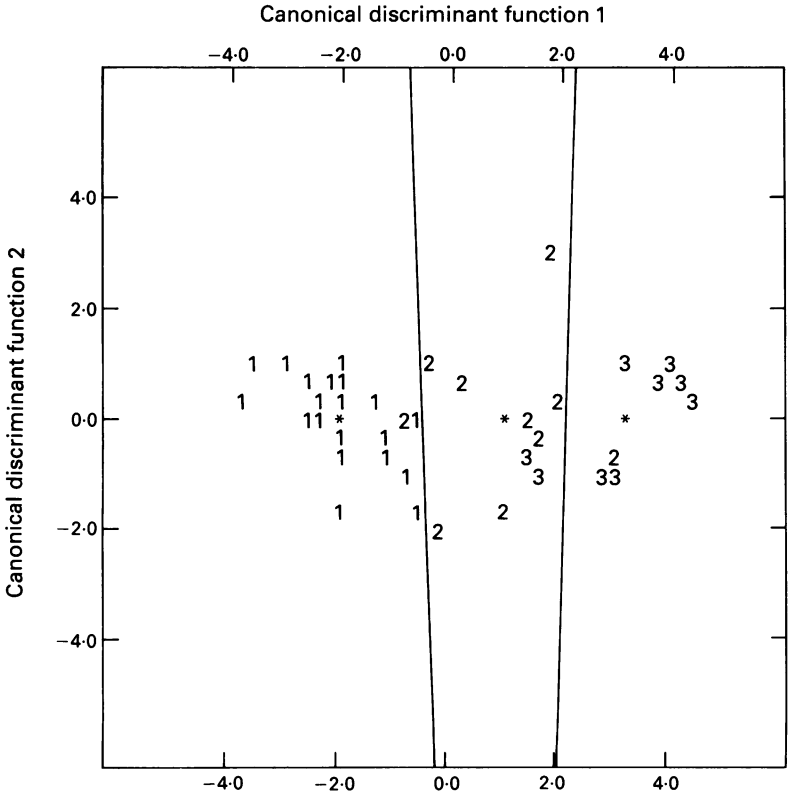


*Figure 5: Canonical analysis of morphometric measurements (intraepithelial lymphocyte count and villous height/ crypt depth ratio in the study groups (see text)). 1=before challenge; 2=group A after challenge; 3=group B after challenge; *=centroid.*

This study confirms previous results indicating the intraepithelial lymphocyte count is the most sensitive morphometric parameter of gluten induced mucosal changes in coeliac disease patients.[2 18 19] A definite increase in the intraepithelial lymphocyte count was indeed found at the end of the challenge in all subjects who had received 100 or 500 mg/day of gliadin. None of the other morphometric variables showed such an unequivocal trend, especially in the patients who had received 100 mg/day of gliadin. Local increase in the number of intraepithelial lymphocytes seems to be an early event in the coeliac lesion (the so called infiltrative lesion).[20] Consequences of the stimulation of the intraepithelial lymphocytes could be cytokine induced crypt hyperplasia, increased epithelial HLA class II expression, and increased epithelial permeability.[21]

Laboratory investigations, such as antigliadin antibody test and the sugar intestinal permeability test, are commonly performed in coeliac disease patients' follow up. Although it is commonly assumed that these tests reflect compliance with the dietary treatment, this has never really been proved for ingestion of small amounts of gluten. In this study the antigliadin antibody-IgG test was often abnormal on gluten free diet and showed almost no correlation with the microchallenge. This result was not unexpected, given the relatively short period these patients had been following the gluten free diet before entering the protocol. Antigliadin antibody-IgG may in fact remain high for years after starting the treatment, due to the 'immunological' memory.[22] Although the serum antigliadin antibody-IgA value was more closely related to the study phase, a negative result could still be found in coeliac disease patients challenged with 500 mg/day of gliadin, a dose corresponding to a 'visible' amount of daily gluten containing food (approximately 12·5 g of wheat flour).

The intestinal permeability test result was even less reliably related to the gliadin intake than the antigliadin antibody test. The reduced predictive power of the former test in treated coeliac disease patients could be explained in different ways. Firstly, intestinal permeability could be primarily abnormal in treated coeliac disease subjects. This possibility, which was suggested some years ago,[23] has recently been reproposed by Hall *et al* who studied the development of gluten sensitive enteropathy in the Irish setter, an animal model of human coeliac disease.[24] With regard to the modest intestinal permeability test-intestinal morphometry relationship, it should be noted that previous attempts to correlate the intestinal permeability test results with jejunal histology have given conflicting results in patients with and without coeliac disease.[25 26] This is not surprising since the test explores the overall mucosal integrity while jejunal histology

evaluates a small fragment of mucosa which is not always representative, particularly when intestinal damage is patchy.

In summary, this study shows that ingestion of small amounts of gliadin over a lengthy period causes dose dependent damage to the small intestinal mucosa in children affected with coeliac disease. Other important variables of this response could be individual factors (for example, age and the HLA haplotype), the type of gluten ingested and the length of the exposure to gluten. The intestinal biopsy specimen with morphometric evaluation of the jejunal mucosa is presently the most accurate method for assessing a patient suspected of poor compliance with the gluten free diet. An increased intraepithelial lymphocyte count should always suggest the possibility of gluten ingestion in treated patients, even when the mucosal structure looks otherwise normal. Although the diagnostic value of both the antigliadin antibody-IgA and the sugar intestinal permeability test is high in patients with active disease, our data seem to indicate that the predictive value of these tests, particularly the intestinal permeability test, is lower in treated patients. Caution is therefore needed when interpreting the results of these investigations in coeliac disease patients who are in long term treatment. The search for accurate, non-invasive tools, for the follow up of treated patients still seems to be needed.

1 Congdon P, Mason MK, Smith S, Crollick A, Steel A, Littlewood J. Small-bowel mucosa in asymptomatic children with celiac disease. *Am J Dis Child* 1981; **135**: 118–21.

2 Leigh RJ, Marsh MN, Crowe P, Kelly C, Garner V, Gordon D. Studies of intestinal lymphoid tissue. IX. Dose-dependent, gluten-induced lymphoid infiltration of coeliac jejunal epithelium. *Scand J Gastroenterol* 1985; **20**: 715–9.

3 Freedman AR, Macartney JC, Nelufer JM, Ciclitira PJ. Timing of infiltration of T lymphocytes induced by gluten into the small intestine in coeliac disease. *J Clin Pathol* 1987; **40**: 741–5.

4 Hamilton JR, McNeil LK. Childhood celiac disease: response of treated patients to a small uniform daily dose of wheat gluten. *J Pediatr* 1972; **81**: 885–93.

5 Packer SM, Charlton V, Keeling JW, *et al*. Gluten challenge in treated coeliac disease. *Arch Dis Child* 1978; **53**: 449–55.

6 Meeuwisse GW. Round table discussion. Diagnostic criteria in coeliac disease. *Acta Paediatr Scand* 1970; **59**: 461–3.

7 Ciclitira PJ, Cerio R, Fagg NLK. Evaluation in coeliac patients of a gluten-free product containing wheat gliadin. *BMJ* 1984; **289**: 83.

8 Ferguson A, Murray D. Quantitation of intraepithelial lymphocytes in human jejunum. *Gut* 1971; **12**: 988–94.

9 Savilahti E, Viander M, Perkkiö M, Vainio E, Kalimo K, Reunala T. IgA antigliadin antibodies: a marker of mucosal damage in childhood coeliac disease. *Lancet* 1983; i: 320–2.

10 Catassi C, Pierani P, Natalini G, Gabrielli O, Coppa GV, Giorgi PL. Clinical application of a simple HPLC method for the sugar intestinal permeability test. *J Pediatr Gastroenterol Nutr* 1991; **12**: 209–12.

11 Penna FJ, Hill ID, Kingston D, Robertson K, Slavin G, Shiner M. Jejunal mucosal morphometry in children with and without gut symptoms and in normal adults. *J Clin Pathol* 1981; **34**: 386–92.

12 Ciclitira PJ, Evans DJ, Fagg NLK, Lennox ES, Dowling RH. Clinical testing of gliadin fractions in coeliac patients. *Clin Sci* 1984; **66**: 357–64.

13 Ciclitira PJ, Cerio R, Ellis HJ, Maxton D, Nelufer J, Macartney JM. Evaluation of a gliadin-containing gluten-free product in coeliac patients. *Hum Nutr: Clin Nutr* 1985; **39c**: 303–8.

14 Scotta MS, De Giacomo C, Maggiore G, Siena S, Ugazio AG. Eucharistic problems for celiac patients. *N Engl J Med* 1982; **30**: 898.

15 Hekkens WTJM. The determination of prolamins in gluten-free food. *Panminerva Med* 1991; **33**: 61–4.

16 Lavö B, Knutson L, Lööf L, Hällgren R. Gliadin challenge-induced jejunal prostaglandin E_2 secretion in coeliac disease. *Gastroenterology* 1990; **99**: 703–7.

17 Lavö B, Knutson L, Lööf L, Odlind B, Hällgren R. Signs of increased leakage over the jejunal mucosa during gliadin challenge of patients with coeliac disease. *Gut* 1990; **31**: 153–7.

18 Schaad UB, Gaze H, Hadorn B. Intraepithelial lymphocytes before and after gluten challenge in children with coeliac disease. *Am J Dis Child* 1981; **135**: 272–3.

19 Montgomery AMP, Goka AKJ, Kumar PJ, Farthing MJG, Clark ML. Low gluten diet in the treatment of adult coeliac disease: effect on jejunal morphology and serum anti-gluten antibodies. *Gut* 1988; **29**: 1564–8.

20 Marsh MN. Grains of truth: evolutionary changes in small intestinal mucosa in response to environmental antigen challenge. *Gut* 1990; **31**: 111–4.

21 Brandtzaeg P, Halstensen TS, Kett K, *et al*. Immunobiology and immunopathology of human gut mucosa: humoral immunity and intraepithelial lymphocytes. *Gastroenterology* 1989; **97**: 1562–84.

22 Troncone R, Ferguson A. Anti-gliadin antibodies. *J Pediatr Gastroenterol Nutr* 1991; **12**: 150–8.

23 Bjarnason I, Peters T, Veall N. A persistent defect in intestinal permeability in coeliac disease demonstrated by a ^{51}Cr-labelled EDTA absorption test. *Lancet* 1983; i: 323–5.

24 Hall EJ, Batt RM. Abnormal permeability precedes the development of a gluten sensitive enteropathy in Irish setter dogs. *Gut* 1991; **32**: 749–53.

25 Nathavitharana KA, Lloyd DR, Raafat F, Brown GA, McNeish AS. Urinary mannitol:lactulose excretion ratios and jejunal mucosal structure. *Arch Dis Child* 1988; **63**: 1054–9.

26 Juby LD, Dixon MF, Axon ATR. Abnormal intestinal permeability and jejunal morphometry. *J Clin Pathol* 1987; **40**: 714–8.