

ORIGINAL ARTICLE

## Enzyme therapy for management of coeliac disease

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### Abstract

**Objective.** Enzyme therapy based on animal digestive extracts was investigated as a means of completely digesting toxic residues from gluten in the small intestine, thus providing a means of protection of the mucosa. **Material and methods.** A randomized, placebo-controlled, clinical trial of an encapsulated enzyme extract was conducted in 21 coeliac patients in remission who were challenged with a modest amount of gluten daily over 2 weeks. Enzyme extract (900 mg) in three divided doses was administered during this challenge to half the group and a placebo to the other half in a double-blind, crossover design. Symptoms were recorded in daily diaries; blood was taken for tissue transglutaminase antibodies (anti-tTG) at the start and at intervals up to 12 weeks. Duodenal biopsies were performed for histological assessment at the start and end of each challenge period for 6 patients chosen at random from volunteers. After a further 10 weeks, the groups were changed over, and the same assessments carried out. **Results.** Only 8 of the 21 patients (38%) had more than 5 episodes of moderate to severe symptoms during either of the gluten challenge periods, and in these, symptoms scores were ameliorated during enzyme therapy compared with the placebo period ( $p < 0.02$ ). Rises of 5 U/ml or more in anti-tTG occurred in only 5 patients at about 6–8 weeks after challenge, but were not correlated with symptoms. Only 1 of the 6 patients had normal histology at entry, thus focusing attention on the need for better management of the disease. By histological criteria, enzyme therapy offered better protection than placebo during the gluten challenges. **Conclusions.** The study supports the use of enzyme supplementation as a safeguard for patients with coeliac disease because of the difficulty of ensuring a strictly gluten-free diet.

**Key Words:** *Animal intestinal extracts, coeliac disease, duodenal biopsy, enzyme therapy, gastrointestinal symptoms, gliadin antibodies, gluten enteropathy, transglutaminase antibodies*

### Introduction

Frazer et al. [1] showed that a toxic peptic-tryptic digest of gluten was rendered non-toxic after further digestion with hog intestinal mucosa, drawing attention to the possibility that an enzyme deficiency was the basic aetiology of coeliac disease (CD).

Bronstein et al. [2] then showed that a peptic-tryptic-pancreatic digest containing even lower molecular weight peptides was still toxic and Cornell & Townley [3] showed that one fraction of this type of digest (Fraction 9) was incompletely digested by duodenal mucosa taken from coeliac patients in remission, and was toxic both *in vitro*, using organ

culture of duodenal mucosa from patients with active CD [4], and *in vivo* to patients with CD. [5]. Further evidence of a peptidase deficiency came when synthetic peptides corresponding to those in Fraction 9 were subjected to further digestion with mucosal homogenates of small intestinal mucosa from coeliac patients in remission. Residues of mainly octapeptides from incomplete digestion of toxic peptides 11–19 and 75–86 of  $\alpha$ -gliadin were obtained [6,7]. These residues corresponded to the structures NPSQQPQ (12–19) and QQPYPQPQ (77–84) of  $\alpha$ -gliadin, respectively, and were both shown to be toxic *in vitro* [8,9] using the foetal

chick assay of Mothes et al. [10]. Further evidence of a peptidase deficiency in CD was provided by Hausch et al. [11] who showed that a bacterial endopeptidase, specifically a prolyl oligopeptidase (EC3.4.2.1.26), reduced the antigenicity of a peptide from  $\alpha_2$ -gliadin, most likely by an attack on the C-terminal side of proline residues in the protein [12].

These observations suggest that, in CD, there is a genetic defect which prevents the biosynthesis of peptidase(s) normally responsible for digestion of specific sequences of amino acids, perhaps including the toxic 4-mer motifs PSQQ and QQQP described by De Ritis et al. [13].

It has become apparent that CD is severely underdiagnosed in many countries. Individuals with CD will be confronted with serious health problems including osteoporosis, lymphomas and some gastrointestinal cancers, unless they are diagnosed early in life and adhere to a strict gluten-free diet. This type of diet is fraught with difficulties and indicates a need for a safeguard that will offset the harmful effects of small amounts of accidentally ingested gluten. To this end, in a randomized controlled trial, we tested a product based on an animal intestinal extract, which contains those enzymes absent or defective in CD [14].

The primary aim of this investigation was to determine whether enzyme therapy could ameliorate symptoms that could be attributed to CD. Secondary aims were to evaluate the effect of the enzyme supplement on coeliac serological responses and duodenal histopathological findings.

### Material and methods

Twenty-one adults (8 M, 13 F, age 18–70 years) with biopsy-proven CD, and on a nominal gluten-free diet, volunteered for the study and gave their written informed consent. All procedures were approved by The Royal Melbourne Hospital Human Research Ethics Committee and were in accordance with the Declaration of Helsinki. The volunteers followed a background diet excluding gluten for the duration of the study. To assess knowledge and compliance to a gluten-free diet, a multilayered assessment tool was used. This included a 2-day qualitative food record, a gluten-specific checklist and a diet interview with a dietitian to ensure that the only gluten ingested was by way of the gluten challenge. All volunteers had a sound understanding of the diet; some required guidance to strengthen compliance in meeting the strict requirement for a gluten-free diet well before the commencement of the trial.

### Gluten challenge

Patients were subjected to a modest gluten challenge consisting of three cracker biscuits (3.5 g each) daily for 14 days, making a total of 13 g of gluten for each part of the trial. The design was a randomized, double-blind crossover one in which half the group was challenged and given the enzyme supplement and the other half challenged and given placebo for the same period (14 days). The active agent was 3 capsules of Glutenon (Glutagen Pty Ltd., Melbourne, Australia, International Patent No PCT/AV03/00633, 2003) taken daily with the biscuit meal and 1 capsule taken with each of the other two meals. The patients recorded symptoms during the challenge as well as over the next 10 weeks.

The groups were then crossed over for the enzyme or placebo interventions and symptoms recorded over the next 12 weeks. The order of interventions was randomized. All symptoms were recorded on a linear scale of units from 0 to 5, where 0 = nil, 1 = slight, 3 = moderate, 5 = severe. Symptoms recorded were fatigue, nausea, vomiting, stomach pain, bloating, cramps, loss of appetite and flatulence.

Patients were also asked to state which of the randomized treatments they thought came first in their particular case.

As well as the above, the number of bowel movements, times of the day and stool consistency on a scale corresponding to the Bristol Stool Form Chart were recorded. None of the patients withdrew from the trial but one patient did not complete the gluten challenge with the placebo because of persistent severe symptoms.

### Tissue transglutaminase antibody (tTG) titres

*IgA-anti tTG antibodies.* Tissue transglutaminase antibody titres were determined in the serum of the blood of all patients at the beginning of each part of the trial, targeting specimens at 0, 1, 2, 4, 6, 8 and 12 weeks. The method used was the purified erythrocyte h-tTG ELISA kit from Inova Diagnostics Inc., San Diego, Calif., USA following the manufacturer's instructions.

### Antigliadin antibody titres

IgA and IgG antigliadin antibody titres were measured in selected serum samples at 0, 2, 4 and 8 weeks in each part of the trial using the ELISA kit from Medical Innovations, Melbourne, Australia, following the manufacturer's instructions.

*Duodenal histopathology*

Duodenal biopsy specimens from 6 unselected consenting patients under 60 years of age were examined by an experienced gastrointestinal pathologist (PSB) as part of the overall assessment of histological changes in duodenal tissue and were based on the Marsh criteria [15]. The effects of the gluten challenges when patients were receiving enzyme therapy were compared with those when patients were taking placebo, requiring 4 biopsies on each of these patients before and after each gluten challenge period (one pair with concurrent Glutenon and the other with placebo).

*Statistical analysis*

The scores used for the symptoms and the crossover design of the trial enabled us to compare the results with placebo and enzyme therapy on the basis of the points scored with each treatment. These differences were then subjected to a paired *t* test, enabling the treatments to be compared for each individual, thus minimizing bias. The estimates of sample size required to halve the total symptom point score (range 5 to 250 points) on enzyme compared to placebo therapy for at least 16 patients was  $n = 21$  at  $p < 0.01$  level of significance (2-tailed test). This allowed for 5 patients to return small negative results for enzyme therapy. The risk of regression towards the mean was difficult to assess, but will be taken into account in further trials.

By using a crossover design, each patient acted as his/her own control in the paired *t* test. In Student's *t* distribution, the sampling behaviour of *t* depends only on  $n$  (the number of patients) and is independent of the values of the true mean and the variance of the sample. Thus, the number of degrees of freedom ( $n - 1$ ) is all important and for a sample size of 21, the change in *p*-values is small for the differences in sample size near this figure.

With respect to paired comparisons made in this study, the Null Hypothesis was that the enzyme supplement and placebo were equally effective and the true average advantage of the enzyme supplement will be zero. The test was therefore to see whether the sample mean is significantly different from zero. Two-tailed tests were used, as these do not assume that the enzyme supplement is superior to the placebo. The statistical power of the trial was difficult to assess because of the uncertainty of the alternative hypothesis that there is a difference in the true means.

The raw data were subjected to the Normal Probability Plot and found not to violate the assumption of data normality ( $p = 0.535$ ). The alpha

value for significance should be 0.05 and the test applied was 2-tailed.

Based on total points scored, the initial analysis gave results that were not statistically significant ( $p < 0.25$ , 2-tailed test) because more than half of the patients were only mildly affected by the gluten challenge. Therefore, it seemed reasonable to consider those patients who were moderately to severely affected at some time during the challenge (symptom points 3–5) since the concept of the product was to prevent troublesome symptoms when gluten was ingested accidentally. The amelioration of the symptoms was thus the primary outcome for statistical analysis, not their elimination.

On this basis, it will be seen that the results are better defined and a subset of 12 patients met these criteria. Histological changes were also analysed in the same way, based on scores for each parameter before and after placebo and enzyme therapy for each of the six patients selected randomly. Paired *t* tests on the serological data were carried out comparing changes in titres on placebo with those on enzyme therapy ( $n = 21$ ).

*Villus height to crypt depth ratio (VH/CR)*. Scores were allocated according to the ratio determined as follows: ratio 5:1–3:1, scored 0; ratio 2:1, scored 1; ratio 1:1–1:2, scored 2; ratio 1:2, scored 3; and ratio 1:4, scored 4.

*Intra-epithelial lymphocytosis (IEL)*. Scores were reported on a scale of 0–3, where normal = 0; mild = 1; moderate = 2; severe = 3.

*Epithelial stunting (ES)*. Scores were reported on a scale of 0–3 where zero = 0; mild = 1; moderate = 2; severe = 3.

*Lamina propria lymphoplasmocytic infiltrate (LPLI)*. Excess infiltrate was scored as normal = 0; mild = 1; moderate = 2; severe = 3.

*Vacuolation of epithelium*. Scores used were zero = 0; mild = 1; severe = 2.

In all cases, damage to the tissue or factors relating to this damage was indicated by the higher scores. Hence any treatment that was beneficial would give lower scores compared with placebo.

## Results

### Symptoms

**Bowel movements.** Bowel movements were very frequent in the worst affected individuals. The four worst affected patients (nos. 6, 15, 19 and 20) registered 52, 30, 55 and 34 bowel movements during the gluten challenge whilst on placebo, compared with 44, 26, 30 and 36 whilst on enzyme therapy. The difference is not significant at  $p=0.05$  probability level (paired  $t$  test, 2-tailed).

**Stool consistency.** Stool consistency did not show any significant difference between placebo and enzyme therapy, with a range of figures between 2.4 and 5.4, with the exception of one patient who had firm stools throughout (1.5–2.1). Scoring of stool consistency can be problematic without accompanying stool weights.

**General symptoms.** On the basis of points for symptoms recorded, there was no significant difference between treatments. The mean points score for all symptoms for patients on placebo was 50.3, compared with 36.9 on enzyme therapy. For the purposes of the study, "reactors" are defined as the participants who developed symptoms during one or other of the gluten challenge periods. However, several of those who had minor reactions to the gluten challenge gave higher results when on enzyme therapy. Figure 1 shows the differences in points for each patient on placebo minus the points whilst on enzyme therapy (P–E). Where more points were scored against the enzyme therapy than the placebo, there were increases in flatulence, loss of appetite, fatigue, abdominal pain and bloating (patients 1, 6, 11, 16 and 18). Of the 21 patients, only 14

experienced at least one daily episode of moderate–severe symptoms, 10 of whom showed a positive benefit from enzyme therapy compared with 4 who showed no benefit ( $p < 0.1$ , paired  $t$  test, 2-tailed). However, 2 patients (nos. 10 and 18) had only single episodes of symptoms of this severity. Taking the remaining 12 most affected patients, 9 were shown to benefit from enzyme therapy, ( $p < 0.05$ , paired  $t$  test, 2-tailed).

Because so many patients were only mildly or not at all affected, it seemed reasonable to analyse those patients who showed moderate to severe symptoms to the challenge whilst on one or other of the treatments. Eight patients showed symptoms of ranking between 3 and 5 on each of several days during either challenge. An amelioration of symptoms was associated with enzyme therapy in 7 of the 8 subjects who had more than 5 episodes where symptoms were  $\geq 3$  on the scale used (Table I). This

Table I. Symptom scores for the 8 patients developing symptoms on gluten challenge (gluten reactors). Episodes of symptoms  $\geq 3$  (moderate–severe).

Patient no.	Placebo	Glutazyme	Difference
6	66	63	3
7	6	0	6
12	14	0	14
13	8	2	6
15	26	9	17
16	0	6	–6
19	50	26	24
20	6	0	6

The remaining 13 patients developed no more than mild symptoms or less than 5 recorded moderate to severe symptoms. Most severely affected – 2 patients (6 and 19).

Abdominal pain, bloating, fatigue and frequent bowel actions were the dominant symptoms in the worst-affected patients.

Only one patient (16) – where difference is negative.

Statistical analysis ( $t$  test)  $p < 0.02$  (paired " $t$ "-test, 2-tailed).

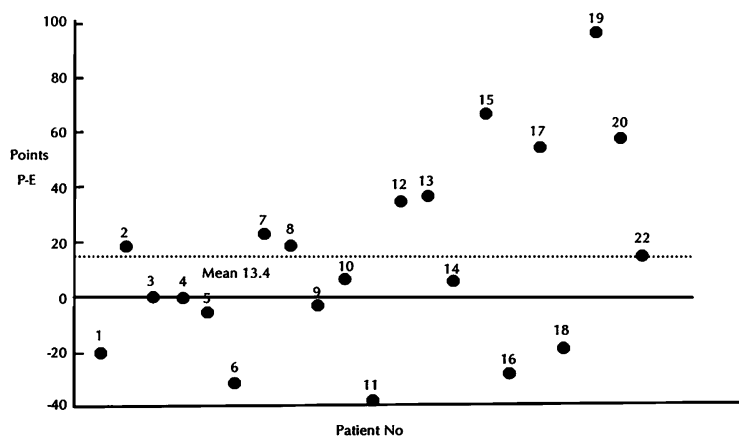


Figure 1. Differences obtained by taking points allocated for symptoms in patients on placebo and subtracting points whilst patients are on enzyme therapy (P–E).

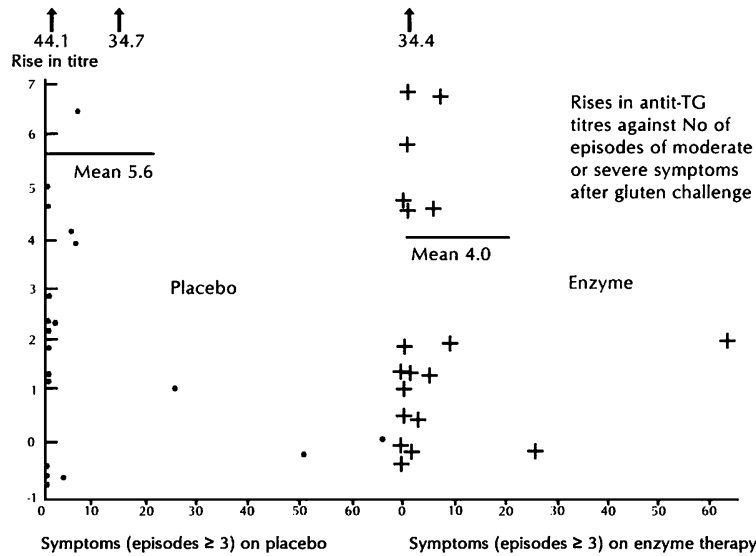


Figure 2. Increases in tTG antibody titres for patients on placebo and enzyme therapy plotted against symptoms (moderate–severe).

result was significant with  $p < 0.02$  (paired  $t$  test, two-tailed).

Symptoms such as abdominal pain and bloating were the most commonly reported, but fatigue, nausea and cramps were also recorded.

Of 16 patients, 9 made a definite judgement about the respective treatment arms; and 7 of these were correct.

*tTG antibodies*

Increases of any significance in titres ( $\geq 5$  U/ml) occurred in only 5 patients and these increases did not correlate with symptoms. Increases occurred in 4

out of the 5 patients when on placebo. These increases were maximal at 3–15 weeks after the challenge. Only one patient (no. 12) had a high titre of 138 U/ml at the start and it remained high throughout the trial. This patient later admitted to regular biscuit snacking. For all patients, the average increase in patients on placebo was 5.6 U/ml, whilst for those on enzyme therapy the average increase was 4.0 U/ml. Of the eight reactors with more than 5 episodes of moderate to severe symptoms, only three gave increases in titres  $\geq 5$  U/ml. One of these patients (no. 16) had a high rise in the titre whilst on placebo, compared with enzyme therapy, yet the symptoms (although only 6 episodes) ran contrary to

Table II. Changes in histological parameters with placebo and enzyme therapy after mild gluten challenge in the 6 patients biopsied.

Patient no.	LPLI		ES	
	Placebo	Enzyme therapy	Placebo	Enzyme therapy
4	0	0	0	0
6	1	1	0	0
12	1	0	1	1
14	1	1	0	1
15	1	0	2	0
19	1	1	1	1
Means <sup>a</sup>	0.83	0.33	0.83	0.50

Abbreviations: LPLI=lamina propria lymphoplasmocytic infiltrate; ES=epithelial stunting.

<sup>a</sup>For intraepithelial lymphocytosis, the means for placebo and enzyme therapy were 0.83 and 0.67, respectively, and for villous height to crypt depth ratio, the means were 0.50 and 0.67, respectively.

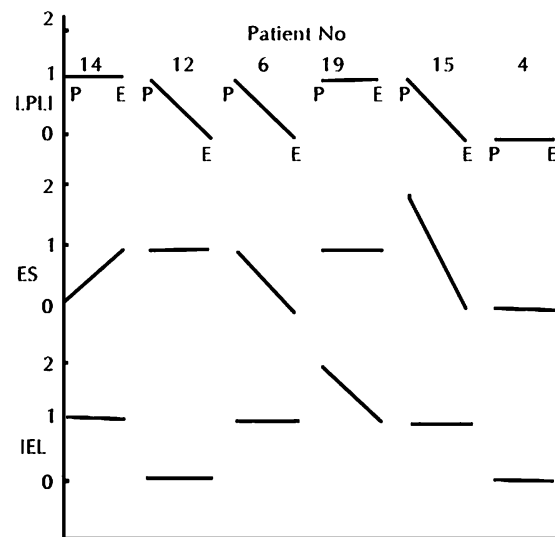


Figure 3. Typical histological changes in patients, by score, after gluten challenge whilst on placebo (P) and enzyme therapy (E).

Table III. Initial titres for anti tTG antibodies compared with initial histology scores for the 6 biopsied patients.

Patient no.	IEL	VH/CR	ES	LPLI	Titre
4	0	1	0	0	6.0
6	1	1	0	1	11.2
12	3	2	2	2	137.7
14	2	2	2	2	16.1
15	1	2	0	1	8.2
19	1	0	0	1	6.2

Abbreviations: LPLI=lamina propria lymphoplasmocytic infiltrate; ES=epithelial stunting; IEL=intraepithelial lymphocytosis; VH/CR=villous height to crypt depth ratio.

Some correlation of initial titres with initial histology (higher scores associated with higher titres).

Patient No. 4 is regarded as being closest to normal histology at the start of the trial.

this trend. Figure 2 shows the increases in all patients on placebo and enzyme therapy.

The average titre over all patients at the beginning of the trial was 16.3 U/ml (range 2.4–138 U/ml). The entry average for the 8 patients who developed symptoms in response to gluten was 28.0 U/ml (range 6.2–138 U/ml), while the 13 who did not react averaged 9.7 U/ml (range 4.2–22.2 U/ml).

### Histology

Small-bowel biopsies from the 6 participants agreeing to biopsy revealed that only 1 patient (No. 4) had normal histology at the beginning of the trial. During gluten challenge, epithelial stunting and lamina propria lymphocytic infiltration were the measures which improved most in association with enzyme therapy versus control. Five out of the 6 people who had biopsies (83%) had noticeable damage at baseline.

In the assessments of lamina propria lymphocytic infiltrate, the ratio of change in the score after gluten challenge compared to before challenge while on placebo was 2.5 times that while on enzyme therapy.

Table IV. Initial histology scores versus antigliadin A/Bs titres.

Patient no.	IEL	VH/CD	ES	LPLI	Starting titre	
					IgA	IgG
4	0	1	0	0	2.3	28.3
6	1	1	0	1	2.7	5.6
12	3	2	2	2	33.3	60.1
14	2	2	2	2	60.7	16.3
15	1	2	0	1	28.0	16.6
19	1	0	0	1	6.3	23.6

Abbreviations: LPLI=lamina propria lymphoplasmocytic infiltrate; ES=epithelial stunting; IEL=intraepithelial lymphocytosis; VH/CR=villous height to crypt depth.

Starting titres tend to be higher in patients with high scores.

IgG titres higher than expected in patients 4 and 19.

Maximum increases in antigliadin titres during gluten challenge (placebo or enzyme therapy) were not informative.

For epithelial stunting, this ratio was 1.7 times. (Table II). Sectioning artefacts, which can compound the assessment of epithelial stunting, would have been randomly distributed between active and placebo interventional periods. Figure 3 shows some typical responses to the gluten challenge. None of these changes reached statistical significance.

Initial titres for tTG antibodies were correlated with initial histology, except for one patient (no. 12) who had high titres throughout the trial. Another patient (no. 14) registered the next highest titre and a patient (no. 4) with the lowest titre had a near normal histology. Three patients with mild scores (nos. 6, 15, 19) had modest titres (Table III).

Increases in tTG antibodies were not correlated with increases in the four main histological parameters. Antigliadin antibody titres were again similar in that initial titres were roughly correlated with the initial histology, with the exception that the IgG result on the patient with normal histology was the second highest result (Table IV). Increases in titres were also not correlated with symptoms of severity  $\geq 3$  (Table V).

Table V. Peak minus baseline antigliadin antibodies (IgA and IgG) in biopsied patients on placebo (P) and enzyme therapy (E) compared with development of symptoms due to gluten challenge, expressed as P–E. Symptoms classified as episodes of moderate–severe ( $\geq 3$  score). Increases in antibodies were generally maximal after 3–8 weeks from start of challenge.

Patient no.	Rise in IgA on P	Rise in IgA on E	Rise in IgG on P	Rise in IgG on E	Rise in episodes of symptoms (P–E)
4	1.0	1.7	0.0	9.7	0
6	0.3	0.1	1.8	0.4	3
12	27.3	79.1	25.1	11.6	14
14	15.5	22.0	24.9	37.3	0
15	3.7	3.7	0.2	6.8	17
19	1.4	0.1	6.4	5.0	24

IgG titres generally higher than IgA titres.

Increases in titres not correlated with increases in episodes of symptoms.

Patients 4 and 14 had no symptoms on P or E.

## Discussion

### *Symptoms*

Bowel movements were frequent in four of the individuals, but together with stool consistency, there were no significant differences between those on placebo and those on enzyme therapy. Patient welfare was of prime concern in estimating the amount of gluten to be used. Even so, 2 patients on placebo became quite ill as a result of the modest gluten challenge and 8 of the 21 patients in the trial had more than 5 episodes of moderate to severe symptoms of any type throughout the clinical trial. This is in keeping with more recent reports which indicate that there is a spectrum of gluten sensitivity [15]. Of these 8 patients, symptoms were ameliorated in 7 during the enzyme therapy compared to placebo and the remaining patient had only 6 episodes of symptoms throughout the period on enzyme therapy. The most common symptom in those reacting to the gluten challenge was not diarrhoea as often reported [16], but abdominal pain and bloating. Fatigue, nausea and cramps were also recorded. In fact, the low level of symptoms in most of the participants during placebo or enzyme therapy limited our capacity to detect any protection by enzyme therapy during gluten challenge as measured by overall symptom scores in the entire cohort. Nevertheless, the points scored for symptoms on placebo were higher than those scored on enzyme therapy, as were the numbers of episodes, but statistical significance was only reached in the 12 patients worst affected by the challenge. At the end of the trial, a blinded review by the patients themselves indicated that those who developed symptoms were less troubled during the period they were on enzyme therapy than during the control period.

The evidence suggests a positive benefit from enzyme therapy when the symptoms from the gluten challenge are more serious and protracted. This is reinforced by the selection of the 8 patients who had 5 or more episodes of moderate–severe symptoms, 7 of whom indicated benefit from enzyme therapy, giving  $p < 0.02$ .

### *tTG antibodies*

At the start of the trial, tTG antibodies were negative ( $< 20$  U/ml) for 17 of the patients, suggesting that these patients were complying with the requirements for a gluten-free diet as judged by transglutaminase antibody measurements. This assay was chosen because of its high sensitivity and specificity [17]. During and after the modest gluten challenge, increases in tTG antibodies of any significance

occurred in only 5 patients and these increases did not correlate with symptoms. There was no strong indication that enzyme therapy attenuated the increases in titres that occurred after challenge with gluten. Patients probably would need to be challenged for a longer period or with larger amounts of gluten before increases in titres become more significant.

### *Histology*

Small-bowel biopsies showed that only 1 patient out of the 6 had normal histology at the beginning of the trial. Epithelial stunting and lamina propria lymphocytic infiltration were less in patients during enzyme therapy compared with when they were on placebo. As the other 5 patients biopsied had noticeable damage at the start of the trial, a need for effective enzyme therapy is indicated, especially as most of these patients had more severe symptoms when taking placebo than when on enzyme therapy.

Epithelial stunting can be difficult to interpret where biopsies are less than ideally sectioned and oriented. Nevertheless, the current study was assessed blindly with respect to intervention period, including randomization of the order of intervention. Thus sectioning artefacts would have been randomly distributed between active and placebo interventional periods. Longer periods of treatment than those used in this trial (2 weeks) may be necessary to confirm the efficacy of enzyme therapy. The changes in histological scores generally were not correlated with moderate to severe symptoms. Only in 3 of the 6 patients biopsied were the changes related to symptoms, reinforcing the finding that symptoms are often not expressed even though damage has occurred [15]. Initial titres for tTG antibodies were correlated to some extent with initial histology. Patients with the highest titres also had the highest histology scores, patients with mild scores had lower titres and the patient with close to normal histology had the lowest titre. However, increases in tTG antibodies were not correlated with increases in the four main histological parameters. The most severely affected patient as judged by IEL and VH/CR, the commonly accepted indicators of damage [16], showed no increase in titre. Increases in antigliadin antibodies were likewise not correlated with symptoms or histological parameters, but again, there was some degree of correlation between the initial titres and the initial histology for each patient. Responses in antigliadin antibodies were marginally better than tTG antibodies in relating to changes in histology and symptoms, but further studies with larger numbers of patients would be required to confirm this.

### General comments

This clinical trial has highlighted a number of important points. The first relates to the wide variation in symptomatic response to gluten. It focuses on the need to improve the diagnosis of CD, which has improved since the introduction of the assays based on tTG antibodies. This is especially important because the majority of individuals with CD remain subclinical or "latent", the mucosal lesion here not being the flat (Type 3) destructive lesion [15]. The histological findings in our small sample support this notion.

The second point relates to the mechanism of damage. It is well known that CD is a consequence of a mucosal cell-mediated response to gluten. Studies with explants of human foetal small bowel maintained in culture [18] have extended our knowledge of these mechanisms, and together with organ culture of explants of foetal chick and coeliac intestine [19,20] they have enabled the structures of the toxic peptides to be elucidated. These types of studies provide the main evidence for the existence of gluten-specific T cells which bring about the secretion of lymphokines into the culture medium, resulting in production of lymphokine-activated killer (LAK) cells which damage small-intestine tissue. All such immune responses may thus be the consequence of the inappropriate response to peptides from cereals which have formed a major part of the human diet only in the last four millennia [21]. Conversely, the finding of sufficient levels of enzymes required to detoxify gluten peptides in the intestine of the cow, pig and sheep [14] is consistent with this concept. If these peptides do not accumulate in the intestine of such animals, the plethora of immunological reactions cannot occur and no adverse effects will be observed. These animals have evolved by natural selection over a much greater period of time than humans, and consequently may have uniformly reached the level of peptidases required for efficient digestion of prolamins-derived peptides which are toxic to humans with CD.

### Patient management

The use of an enterically coated capsule containing an animal intestinal extract, which contains those enzymes absent or defective in CD [14], supports the use of this type of product for management of this disease. The majority of patients biopsied at the beginning of the trial showed small-bowel abnormalities which indicated that traces of gluten were present in their diet, even though they were taking all reasonable precautions to avoid gluten. This therapy will reduce the hardship associated with maintaining a gluten-free diet in a variety of contemporary

culinary circumstances, and should ensure that symptoms are minimized, small-bowel histology normalized and coeliac complications reduced.

Progress in tissue repair in a newly diagnosed patient with CD after introduction of a gluten-free diet could be followed by the serological tests used in this study. Even though there were no definite movements in antibody titres during the short, modest, gluten challenge employed in this study, the correlation between initial histology and antibody titres warrants further study. These results must be considered as being of a preliminary nature and a trial using longer periods of treatment should provide evidence of the long-term benefits of enzyme therapy as a safeguard against inadvertent gluten ingestion.

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