A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease[1-3]

Carlo Catassi, Elisabetta Fabiani, Giuseppe Iacono, Cinzia D'Agate, Ruggiero Francavilla, Federico Biagi, Umberto Volta, Salvatore Accomando, Antonio Picarelli, Italo De Vitis, Giovanna Pianelli, Rosaria Gesuita, Flavia Carle, Alessandra Mandolesi, Italo Bearzi, and Alessio Fasano

ABSTRACT

Background: Treatment of celiac disease (CD) is based on the avoidance of gluten-containing food. However, it is not known whether trace amounts of gluten are harmful to treated patients.

Objective: The objective was to establish the safety threshold of prolonged exposure to trace amounts of gluten (ie, contaminating gluten).

Design: This was a multicenter, double-blind, placebo-controlled, randomized trial in 49 adults with biopsy-proven CD who were being treated with a gluten-free diet (GFD) for ≥ 2 y. The background daily gluten intake was maintained at <5 mg. After a baseline evaluation (t_0), patients were assigned to ingest daily for 90 d a capsule containing 0, 10, or 50 mg gluten. Clinical, serologic, and histologic evaluations of the small intestine were performed at t_0 and after the gluten microchallenge (t_1).

Results: At t_0, the median villous height/crypt depth (Vh/Cd) in the small-intestinal mucosa was significantly lower and the intraepithelial lymphocyte (IEL) count ($\times 100$ enterocytes) significantly higher in the CD patients (Vh/Cd: 2.20; 95% CI: 2.11, 2.89; IEL: 27; 95% CI: 23, 34) than in 20 non-CD control subjects (Vh/Cd: 2.87; 95% CI: 2.50, 3.09; IEL: 22; 95% CI: 18, 24). One patient (challenged with 10 mg gluten) developed a clinical relapse. At t_1, the percentage change in Vh/Cd was 9% (95% CI: 3%, 15%) in the placebo group ($n = 13$), −1% (−18%, 68%) in the 10-mg group ($n = 13$), and −20% (−22%, −13%) in the 50-mg group ($n = 13$). No significant differences in the IEL count were found between the 3 groups.

Conclusions: The ingestion of contaminating gluten should be kept lower than 50 mg/d in the treatment of CD. *Am J Clin Nutr* 2007; 85:160−6.

KEY WORDS Gastroenterology, celiac disease, gluten toxicity, small-intestinal morphometry, gluten-free diet, gluten threshold in gluten-free food

INTRODUCTION

Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten—the major protein fraction contained in the cereals wheat, rye, and barley—in genetically susceptible persons. CD is a life-long disorder affecting 0.5–1% of the general population worldwide. The standard treatment of CD involves the consumption of a diet completely devoid of gluten proteins, a so-called gluten-free diet (GFD). In the long term (1–2 y), a GFD is associated with clinical, serologic, and histologic remission (1). However, it is almost impossible to maintain a diet with a zero gluten content because gluten contamination is very common in food. "Hidden" gluten (used as a protein filler) may be found in commercially available products, such as sausages, soups, soy sauces, and ice cream. Even products specifically targeted to dietary treatment of CD may contain tiny amounts of gluten proteins, either because of the cross-contamination of originally gluten-free cereals during their milling, storage, and manipulation or because of the presence of wheat starch as a major ingredient.

The potential toxicity of trace amounts of gluten is still unclear. We previously showed in treated CD patients that the 4-wk ingestion of 100–500 mg gliadin/d (roughly equivalent to 200–1000 mg gluten) is able to cause measurable changes in the architecture of the small-intestinal mucosa (2). Only limited data are available on the toxicity of lower doses of gluten (3–6). This is an important issue because the daily ingestion of contaminating gluten in apparently well-treated CD patients is most likely to range from 5 to 50 mg.

Establishing a safe threshold of gluten consumption for CD patients is a matter of major public health importance, particularly in light of the recent reports concerning the high prevalence

of the disease worldwide (7, 8). The recent National Institutes of Health Consensus Conference position on CD estimated that as many as 3 million people in the United States are affected by CD. These findings, together with the recently approved Food Allergen Labeling and Consumer Protection Act, created a vacuum in terms of health care policy, food safety, legislative guidelines, and industry-related legal liability that needs to be filled to allow the Food and Drug Administration's governmental mandate to implement the new bill by 2006. The "gluten threshold" topic is currently under evaluation by the Codex Alimentarius, the WHO/FAO commission that is in charge of setting food standards at the international level. To address the aforementioned issues we undertook a prospective, double-blind, placebo-controlled multicenter trial to investigate the toxicity of gluten traces in the celiac diet, with the cooperation and the sponsorship of the Italian Celiac Society (Associazione Italiana Celiachia; AIC). We report herein the final results of this study.

SUBJECTS AND METHODS

Study design

This was a prospective, multicenter, placebo-controlled, double-blind, randomized trial performed between the years 2001 and 2004. The patients were adults with biopsy-proven CD who had consumed a GFD for ≥ 2 y and were in apparent good health. Patients were excluded if they 1) were younger than 18 y, 2) were poorly compliant with the GFD, 3) were positive for anti-tissue transglutaminase (tTG) antibodies or had a villous height/crypt depth (Vh/Cd) <1.5 at baseline (t_0), or 4) associated conditions, such as selective immunoglobulin A (IgA) deficiency or other autoimmune diseases. Patients that qualified for the study were interviewed and gave their informed consent. Control subjects (only for comparison of the morphometric values at t_0) were adults who were negative for serologic CD markers and for *Helicobacter pylori* (urea breath test) and were undergoing upper endoscopy for diagnostic purposes.

The patients qualifying for the trial underwent a screening and a dietary interview (t_{-1}). They were asked to maintain a strict GFD during the study period, ie, avoidance of any possible source of gluten contamination (such as restaurant meals). The only cereal-based food they were allowed to eat was the special GFD products on the market in Italy, which Italian law establishes as having a gluten contamination of <20 ppm (20 ppm = 20 mg/kg product). After 1 mo the subjects returned for a baseline evaluation (t_0), which involved 1) a clinical examination, 2) a dietary interview, 3) blood collection for serum anti-tTG antibody and antigliadin antibody (AGA) measurements, and 4) an endoscopy and small-intestinal biopsy. While still adhering to a strict GFD, the patients were randomly assigned (by the coordinating center) to ingest daily and for 90 d a capsule containing either 10 mg purified gluten, 50 mg purified gluten, or 50 mg cornstarch as a placebo (double-blind microchallenge). After completing the 3-mo microchallenge (t_1), the patients repeated the same clinical, serologic, and histologic tests as at t_0. If any patient had symptoms suggestive of CD relapse during the microchallenge, the protocol was stopped and the patient was asked to perform the t_1 evaluation before dropping out of the study. From t_{-1} to t_1, the adherence to both the GFD and the study protocol and clinical progress were checked weekly by telephone interview. The study protocol was approved by the Ethical Committee of the Università Politecnica delle Marche, Ancona, Italy.

Methods

Purified gluten was used for the preparation of the capsules (Amygluten 110; Tate & Lyle PLC, London, United Kingdom). We used whole gluten rather than single gluten fractions because it has been shown that not only gliadins, but also glutenins (the other major component of gluten), contain toxic epitopes (9). Gelatin capsules that would quickly dissolve in the stomach were prepared by the pharmacy of the coordinating center. On a dry weight basis, the capsules contained 10 mg raw gluten, 50 mg raw gluten, or 50 mg cornstarch (placebo). All laboratory tests and analyses of biopsy specimens were centrally performed at the Università Politecnica delle Marche. Serum IgG class AGA and IgA class anti-tTG were measured by standard enzyme-linked immunosorbent assay methods (Alfa-Gliatest and h-TTG; Eurospital Trieste, Trieste, Italy).

Small-bowel biopsies (≥ 4 specimens from each procedure) were taken from the second part of the duodenum. All biopsy specimens were oriented and fixed in 10% formalin, embedded in paraffin wax. The sections (5 μm) were stained with hematoxylin and eosin and immunostained by using anti-human CD3 antibody (DAKO, Glostrup, Denmark) to enhance diagnostic accuracy in counting intraepithelial lymphocytes (IELs). Only well-oriented sections were examined; when necessary, the samples were dissected again until they were of good quality. The specimens were examined in batches by 2 pathologists with long-standing experience in morphometric analysis (IB and AM), who were blinded to subject assignment. The morphometric analysis of the sections was performed on ≥ 10 well-oriented villi, arranged like fingers, and 10 well-oriented crypts, arranged perpendicular to the muscolaris mucosae, by a computerized image analyzer (IBAS-AT; Kontron, Munich, Germany). The following variables were evaluated: Vh/Cd and IEL count. The number of IELs was calculated by computing their mean value out of 1000 enterocytes and expressed as a percentage of enterocytes in the area analyzed (2). The IEL count was performed in well-oriented villi through the overall length of the villi, including the villous tip. The overall architecture of the small-intestinal mucosa was also evaluated according to the Marsh-Oberhuber classification (10).

Analysis of background gluten intake

The consumption of gluten-free flour was measured in a different sample of 46 adults with CD (30 women, 16 men; mean age: 36 y; range: 19–63 y) who had been consuming a GFD long term (>2 y). These subjects were asked to report the type and the amount of all the special gluten-free products consumed during 30 consecutive d in a diary. A random sample of 42 gluten-free products consumed by these subjects was collected and analyzed for gluten contamination by enzyme-linked immunosorbent assay (Ridascreen Gliadin; R-Biopharm AG, Darmstadt, Germany) with a sensitivity limit of 3 ppm of gluten.

Statistical analysis

The sample size was selected to detect a mean effect size of 0.3 for changes in morphometric variables before and after the microchallenge, which was expressed as a percentage of baseline values among the 3 groups of patients at a significance level of

5% and a power of 85%; one-way analysis of variance was used for the data analysis. The number of patients required was 39 ($n = 13$ for each group). Considering a 20% dropout rate, 49 subjects were recruited. The normality of the distribution of the examined variables was assessed by using the Shapiro-Wilk test. Nonparametric methods were used for the statistical analysis of the data because the morphometric variables were not normally distributed. A nonparametric analysis of variance (Kruskal-Wallis test) was performed to evaluate the treatment groups (placebo, 10 mg/d, and 50 mg/d) at t_0. Wilcoxon's test was used to compare pooled CD patients with control subjects at t_0. The correlation between Vh/Cd and IELs was analyzed by Spearman's coefficient. Changes in morphometric variables before and after the microchallenge were expressed as percentages of the t_0 values. The comparisons between the 3 groups were performed by the Kruskal-Wallis test. Because this test has an asymptotic efficiency of 96% with respect to the one-way parametric analysis of variance (11), the number of patients recruited guaranteed a level of study power not lower than 80%. The results were expressed as medians and 95% CIs. A 5% probability was used to assess statistical significance.

RESULTS

Forty-nine subjects were enrolled in the microchallenge study: 37 women and 12 men with a median age of 30.5 y (range: 19.8–55.4 y) and a median BMI of 21.9 (range: 16.8–30.1). After t_0, 7 of the 49 patients had to be excluded because of abnormal small-intestinal histology (4), development of thyroid carcinoma (1), or gastric polyposis confirmed by gastroduodenoscopy (1) or because the subject refused randomization (1). Three other cases did not complete the microchallenge because of change of residence (1 challenged with 50 mg), poor adherence to the protocol (1 challenged with 10 mg), or development of symptoms (1 challenged with 10 mg). Thirty-nine patients completed the study protocol: 30 women and 9 men with a median age of 30.6 y (range: 19.8–55.4 y) and who had consumed a GFD for a median of 10.1 y (range: 2.1–28.0 y). Of these 39 subjects, 13 were challenged with placebo, 13 with 10 mg gluten/d, and 13 with 50 mg gluten/d.

The control subjects were 20 adults ($n = 13$ women and 7 men) with a median age of 34.5 y (range: 24.0–60.0 y) and a final diagnosis of functional dyspepsia ($n = 13$) or gastroesophageal reflux ($n = 7$).

Clinical and serologic evaluation

One patient challenged with 10 mg gluten/d showed typical signs of relapse (vomiting, diarrhea, and abdominal distension) after 6–8 wk of microchallenge but refused to repeat the t_1 evaluation. The comparison between the baseline and the post-challenge findings in the 39 patients completing the microchallenge did not show any significant changes in the clinical outcome between the 3 groups. The results of the serologic CD markers are shown in **Figure 1**. In all patients, the IgA anti-tTG and IgG AGA values were in the normal range, both at baseline and after the microchallenge. No significant difference was detected between the 3 groups at baseline (Kruskal-Wallis test; $P = 0.30$ and $P = 0.45$, respectively). After the micro-challenge the IgA anti-tTG did not show any significant change ($P = 0.12$), while the IgG-AGA showed a significant decrease in the group
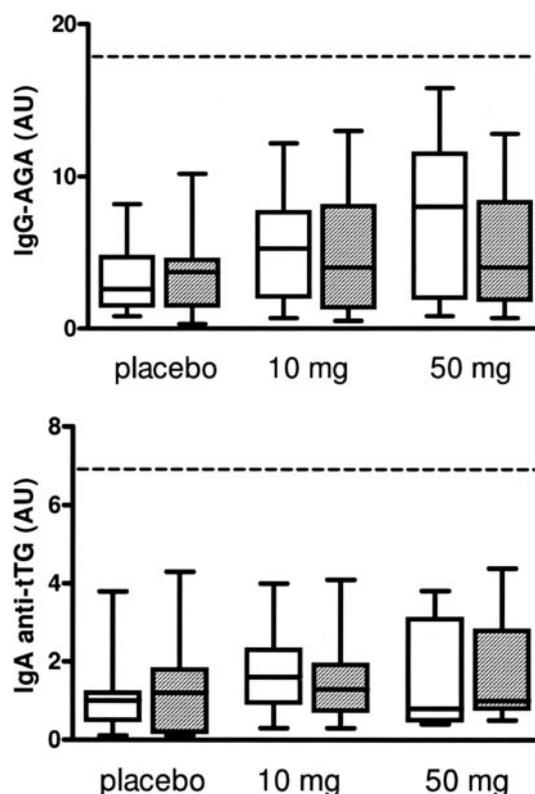

FIGURE 1. Median and interquartile concentrations of serum immunoglobulin A (IgA) class anti-tissue transglutaminase (tTG) and IgG class anti-gliadin (AGA) antibodies before (□) and after (▨) the gluten challenge in the 3 study groups: placebo ($n = 13$), 10 mg gluten/d ($n = 13$), and 50 mg gluten/d ($n = 13$). No significant differences at baseline and no significant increase after the microchallenge were detected (Kruskal-Wallis test). The dashed line represents the normal cutoff. AU, arbitrary units.

challenged with 50 mg of gluten in comparison with the placebo ($P = 0.04$).

Small-bowel biopsy results

The results of the small-intestinal morphometric measures in CD patients and control subjects at baseline are shown in **Table 1**. The Vh/Cd ratio was significantly lower in CD patients than in control subjects, and the IEL count was significantly higher in CD patients than in control subjects. In the pooled CD group ($n = 39$), the correlation between the Vh/Cd and the IEL count was

TABLE 1
Intestinal morphometric evaluation of the subjects before and after the gluten microchallenge[1]

	Control subjects ($n = 20$)	CD patients at baseline ($n = 39$)	P^2
Villous height (μm)	372.7 (339.5, 385.4)	354.3 (318.0, 380.5)	0.130
Crypt depth (μm)	135.1 (124.8, 149.7)	150.9 (134.9, 160.6)	0.077
Vh/Cd	2.9 (2.5, 3.1)	2.2 (2.1, 2.9)[3]	0.019
IEL count ($\times 100$ enterocytes)	22 (18, 24)	27 (23, 34)[3]	0.002

[1] All values are medians; 95% CIs in parentheses. CD, celiac disease; Vh/Cd, villous height/crypt depth; IEL, intraepithelial lymphocyte.
[2] The Wilcoxon test was used for the comparison between groups.
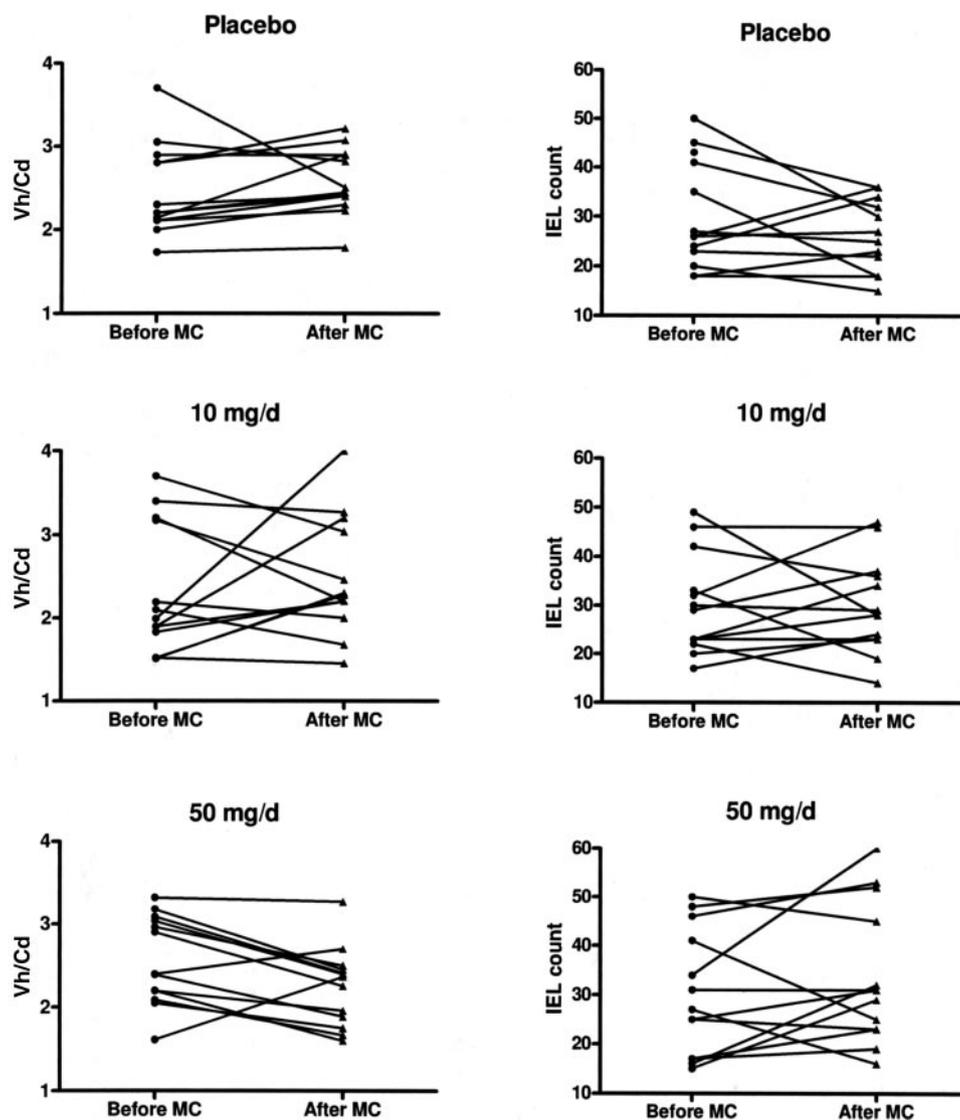[3] Significantly different from the control subjects, $P < 0.05$.

FIGURE 2. Individual results of the small-intestinal morphometric evaluation before and after the 3-mo gluten microchallenge (MC) in the 3 study groups: placebo ($n = 13$), 10 mg gluten/d ($n = 13$), and 50 mg gluten/d ($n = 13$). A significant difference in villous height/crypt depth (Vh/Cd) was observed between the placebo and the 50-mg groups (Kruskal-Wallis test, $P = 0.029$). No significant differences in intraepithelial lymphocyte (IEL) ratio were detected between the 3 groups.

weak and not statistically significant at either baseline ($r = -0.31$; 95% CI: $-0.56, 0.02$; $P = 0.06$) or after the microchallenge ($r = -0.29$; 95% CI: $-0.55, 0.03$; $P = 0.07$).

The results of the gluten microchallenge are shown in **Figure 2** and **Table 2**. No significant difference in the morphometric indexes was found at baseline between groups. After the microchallenge, the Vh/Cd index improved in 11 of 13 subjects (85%; 95% CI: 55%, 98%) in the placebo group, in 6 of the 13 subjects (46%; 95% CI: 19%, 75%) in the 10-mg group, and in only 2 of the 13 subjects (15%; 95% CI: 2%, 45%) in the 50-mg group; the difference between the 50-mg group and the placebo group was significant. A significant improvement in the median percentage variation between t_1 and baseline (percentage change) of the Vh/Cd index was observed in the placebo group but not in the 10-mg and 50-mg groups. Conversely, at t_1, the Vh/Cd index showed a significant decrease in the group challenged with 50 mg

gluten/d. The Kruskal-Wallis test showed a significant difference in the percentage change in Vh/Cd between the placebo and the 50-mg group ($P = 0.029$). An increase in IELs was observed in only 5 of the 13 subjects (38%; 95% CI: 14%, 68%) in the placebo group, in 6 of the 13 subjects (46%; 95%: CI 19%, 75%) in the 10-mg group, and in 8 of the 13 subjects (61%; 95% CI: 32%, 86%) in the 50-mg group. No significant differences in the percentage change in IEL counts were found between the 3 groups.

Analysis of background gluten intake

The daily consumption ($\bar{x} \pm SD$) of commercially available gluten-free products was 332 ± 98 g (range: 177–574 g) in 46 adults being treated for CD. The median gluten content of the 42 gluten-free products analyzed was 5.2 ppm (ppm = mg/kg) (range: 0–50 ppm). On the basis of these values, we estimated

TABLE 2
Results of the 3-mo gluten microchallenge in patients with celiac disease[1]

	Group			
	Placebo (n = 13)	10 mg gluten/d (n = 13)	50 mg gluten/d (n = 13)	P
Marsh-Oberhuber grading of small-bowel histology[2]				
Baseline				
0	8	8	7	0.263[3]
1	4	1	5	
2	1	4	1	
After microchallenge				
0	8	8	5	0.433[3]
1	4	5	5	
2	1	0	3	
Intestinal morphometric results at baseline[4]				
Villous height (μm)	354 (315, 437)	318 (306, 366)	380 (266, 396)	0.431[5]
Crypt depth (μm)	150 (134, 165)	161 (124, 187)	131 (110, 159)	0.197[5]
Vh/Cd	2.2 (2.1, 3.0)	2.0 (1.8, 3.4)	2.4 (2.2, 3.2)	0.273[5]
IEL count ($\times$ 100 enterocytes)	26 (23, 45)	29 (23, 46)	27 (17, 48)	0.968[5]
Morphometric changes after the microchallenge (%)[4]				
Villous height	5 (−5, 13)	9 (−9; 19)	−17 (−30, 23)	0.198[5]
Crypt depth	−2 (−16, 9)	3 (−28, 21)	3 (−17, 24)	0.693[5]
Vh/Cd	9 (3, 15)	−1 (−18, 68)	−20 (−22, −13)[6]	0.029[5]
IEL count	−4 (−22, 42)	0 (−14, 47)	12 (−8, 93)	0.514[5]

[1] n = 39. Vh/Cd, villous height/crypt depth; IEL, intraepithelial lymphocyte.

[2] 0 = normal, 1 = infiltrative lesions (increased IEL count), 2 = hyperplasic lesions (increased IEL count and increased crypt depth).

[3] Fisher's exact test.

[4] All values are medians; 95% CIs in parentheses.

[5] Kruskal-Wallis test.

[6] Significantly different from placebo, $P < 0.05$.

that the background gluten intake from the GFD followed by our patients during the microchallenge study was <5 mg/d.

DISCUSSION

The morphometric analysis of the small-intestinal mucosa, particularly the evaluation of the Vh/Cd index and the IEL count, is the reference method for the quantitative assessment of gluten-induced damage in CD. The normal values found in our control subjects agree with previous data for both Vh/Cd and the IEL count (5). The IEL count is considered to be the most sensitive index of the celiac enteropathy (12). However, this paradigm has been investigated mainly in active CD but does not necessarily apply to CD patients receiving long-term dietary treatment. In our study, the analysis of the effects of the gluten microchallenge indeed suggested that the Vh/Cd index reflected more closely the damage induced by traces of gluten in the diet than did the IEL count.

Despite the restricted criteria adopted in this study, the baseline duodenal biopsy results showed evidence of histologic damage (decreased median Vh/Cd and increased median IEL count) in adult CD patients receiving long-term dietary treatment. Furthermore, 4 of 49 subjects had to be excluded from the protocol because severe enteropathy (obscuring the possible effects of the gluten microchallenge) was detected at the baseline evaluation. These results confirm that an abnormal small-bowel morphology persists in a significant proportion of CD patients treated with a GFD, despite full resolution of their symptoms (13–15). This finding has been shown to be related to the ongoing ingestion of

gluten, either deliberate or inadvertent, causing persistent inflammation in the small-intestinal mucosa (14, 16). This interpretation is supported by our results because we noticed a significant improvement in the intestinal architecture (Vh/Cd index) in the placebo group after 3 mo, as a consequence of the strict monitoring of the GFD imposed by the study protocol. On the other hand, neither the IgA class anti-tTG nor the IgG class AGA antibodies (which were always normal) showed a correlation with the mucosal changes found in some patients, confirming that these serologic markers are not sensitive enough to detect the residual enteropathy that can be found in apparently healthy CD patients receiving treatment with a GFD (17, 18).

The gluten microchallenge disclosed large interpatient variability in the sensitivity to gluten traces. Some CD patients showed a clear-cut worsening of the small-intestinal architecture after ingesting only 10 mg gluten/d, whereas others had an apparent improvement in mucosal histology after the 3-mo challenge with 50 mg gluten/d (Figure 2). Furthermore, one patient challenged with 10 mg gluten/d experienced clinical symptoms after a few weeks, whereas none of the 13 subjects receiving 50 mg gluten/d had clinical evidence of relapse. Despite this wide individual variability, which must be taken into account when implementing a threshold that is safe for all patients, we showed that 50 mg gluten/d, if introduced for 3 mo, were sufficient to cause a significant decrease in the Vh/Cd index in treated CD patients. This is an important new finding that must be interpreted with caution, for several reasons. Because of the limited number of patients, we were not able to reach firm conclusions about the potential toxicity of 10 mg gluten/d, which remained a "gray"

area. For ethical reasons we had to limit the duration of the microchallenge to 3 mo, but it is well known that mucosal deterioration may become manifest after a longer gluten challenge (19). Reactions to gluten are not only influenced by the quantity but also the quality of the ingested protein, which may change according to cereal variety (20) and food processing (raw versus cooked grain, fermentation, etc) (21).

The effects of a low gluten intake in CD patients have been investigated in a limited number of studies. Ciclitira et al (22) analyzed the toxicity and time response of a gliadin dose (the major toxic fraction of gluten) in a single patient. They concluded that 10 mg produced no change, 100 mg a very slight measurable change, 500 mg a moderate change, and 1 g extensive damage to small-intestinal morphology. The same group also reported that the ingestion of 2.4–4.8 mg gluten/d caused no change in the jejunal biopsy morphometry of treated CD patients after either 1 or 6 wk (3, 23). Ejderhamn et al (4) showed that a daily intake of 4–14 mg gliadin did not affect the morphology of the small-bowel mucosa in CD patients receiving long-term treatment with a GFD. Recent Finnish studies indicate that an intake of 20–36 mg gluten/d has no detectable effect on mucosal histology (5, 6). We previously showed that a 4-wk challenge with 100 mg gliadin/d caused deterioration of the small-intestinal architecture and that the histologic changes were more pronounced in patients challenged with 500 mg gliadin/d (2). Finally, a higher gluten intake (1–5 g/d), still lower than the normal gluten intake for the non-CD population in Western countries (10–20 g/d) (24), caused relapse of disease at a clinical, laboratory, and histologic level, both in children and in adults (25–27). On the basis of the evidence from the current study and the quoted literature, it appears that 50 mg gluten/d is the minimum dose required to produce measurable damage to the small-intestinal mucosa in CD patients.

Currently, different national positions hamper the implementation of uniform guidelines on the maximum level of gluten contamination (expressed as ppm) that can be tolerated in products that are marketed for the treatment of CD. This is a "hot" topic that was recently reviewed extensively (28, 29). In Northern European countries, up to 200 ppm gluten is permitted in food for CD patients, to use wheat starch as ingredient. Conversely, a more prudent value of 20 ppm has been adopted in North American and southern European countries. On the basis of their clinical and analytic data, Finnish experts recently advocated the intermediate limit of 100 ppm (30). The decision about what the threshold is depends, however, not only on the minimum toxic dose but also on the amount of gluten-free products consumed. Our results indicate that 200 ppm is not a safe threshold because the harmful gluten intake of 50 mg/d could be reached even with a moderate consumption (≥250 g/d) of nominally gluten-free products. A 100-ppm threshold, by allowing up to 10 mg gluten/100 g product, is also probably not suitable for generalized use, especially in countries such as Italy, where consumption of wheat substitutes is occasionally as high as 500 g/d (as shown by our data). The threshold of 20 ppm keeps the intake of gluten from "special celiac food" well below the amount of 50 mg/d, which allows a safety margin for the variable gluten sensitivity and dietary habits of patients.

In conclusion, this study confirmed that an abnormal small-bowel morphology persisted in a significant proportion of CD patients being treated with a GFD, most likely because of the persistent ingestion of trace amounts of gluten. The protracted intake of 50 mg gluten/d produced significant damage in the architecture of the small intestine in patients being treated for CD. However, the sensitivity to trace intakes of gluten showed large interpatient variability, a feature that should be accounted for in the implementation of a safe gluten threshold. These findings should be confirmed by further studies in larger numbers of CD patients. Finally, the relation between the intestinal damage induced by trace intakes of gluten and the long-term complications of CD remains to be elucidated. ☒

REFERENCES

1. Fasano A, Catassi C. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. Gastroenterology 2001;120:636–51.
2. Catassi C, Rossini M, Rätsch IM, et al. Dose dependent effects of protracted ingestion of small amounts of gliadin in coeliac disease children: a clinical and jejunal morphometric study. Gut 1993;34:1515–9.
3. Ciclitira PJ, Ellis HJ, Fagg NL. Evaluation of a gluten free product containing wheat gliadin in patients with coeliac disease. Br Med J 1984;289:83.
4. Ejderhamn J, Veress B, Strandvik B. The long term effect of continual ingestion of wheat starch-containing gluten-free products in celiac patients. In: Kumar PJ, ed. Coeliac disease: one hundred years. Leeds, United Kingdom: Leeds University Press, 1988:294–7.
5. Kaukinen K, Collin P, Holm K, et al. Wheat starch-containing gluten-free flour products in the treatment of celiac disease and dermatitis herpetiformis. A long-term follow-up study. Scand J Gastroenterol 1999;34:909–14.
6. Peräaho M, Kaukinen K, Paasikivi K, et al. Wheat-starch based gluten-free products in the treatment of newly detected coeliac disease. Prospective and randomised study. Aliment Pharmacol Ther 2003;17:587–94.
7. Mäki M, Mustalahti K, Kokkonen J, et al. Prevalence of celiac disease among children in Finland. N Engl J Med 2003;348:2517–24.
8. Fasano A, Berti I, Gerarduzzi T, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med 2003;163:286–92.
9. Vader W, Kooy Y, Van Veelen P, et al. The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. Gastroenterology 2002;122:1729–37.
10. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol 1999;11:1185–94.
11. Siegel S, Castellan NJ. Nonparametric statistics for the behavioural sciences. New York, NY: McGraw Hill, 1988.
12. Järvinen TT, Collin P, Rasmussen M, et al. Villous tip intraepithelial lymphocytes as markers of early-stage coeliac disease. Scand J Gastroenterol 2004;39:428–33.
13. Lee SK, Lo W, Memeo L, Rotterdam H, Green PH. Duodenal histology in patients with celiac disease after treatment with a gluten-free diet. Gastrointest Endosc 2003;57:187–91.

14. Cummins AG, Thompson FM, Butler RN, et al. Improvement in intestinal permeability precedes morphometric recovery of the small intestine in celiac disease. Clin Sci 2001;100:379–86.

15. Collin P, Mäki M, Kaukinen K. Complete small intestinal mucosal recovery is obtainable in the treatment of celiac disease. Gastrointest Endosc 2004;59:158–9.

16. Murray JA. Celiac sprue: to diet or digest. Clin Gastroenterol Hepatol 2005;3:629–30.

17. Vahedi K, Mascart F, Mary JY, et al. Reliability of antitransglutaminase antibodies as predictors of gluten-free diet compliance in adult celiac disease. Am J Gastroenterol 2003;98:1079–87.

18. Kaukinen K, Sulkanen S, Mäki M, Collin P. IgA-class transglutaminase in evaluating the efficacy of gluten-free diet in coeliac disease. Eur J Gastroenterol Hepatol 2002;14:311–5.

19. Högberg L, Stenhammar L, Wagermark J. Very late mucosal relapse in a girl with celiac disease. Acta Paediatr 1993;82:887–9.

20. Molberg O, Uhlen AK, Jensen T, et al. Mapping of gluten T-cell epitopes in the bread wheat ancestors: implications for celiac disease. Gastroenterology 2005;128:393–401.

21. Di Cagno R, De Angelis M, Auricchio S, et al. Sourdough bread made from wheat and nontoxic flours and started with selected lactobacilli is tolerated in celiac sprue patients. Appl Environ Microbiol 2004;70:1088–96.

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

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

24. van Overbeek FM, Uil-Dieterman IGA, Mol IW, Köhler-Brands L, Heymans HAS, Mulder CJJ. The daily gluten intake in relatives of patients with celiac disease compared with that of the general Dutch population. Eur J Gastroenterol Hepatol 1997;9:1097–9.

25. Laurin P, Wolving M, Fälth-Magnusson K. Even small amounts of gluten cause relapse in children with celiac disease. J Pediatr Gastroenterol Nutr 2002;34:26–30.

26. Jansson UHG, Gudjonsdottir AH, Ryd W, Kristiansson B. Two different doses of gluten show a dose-dependent response of enteropathy, but not of serological markers during gluten challenge in children with celiac disease. Acta Paediatr 2001;90:255–9.

27. Montgomery AM, Goka AK, Kumar PJ, Farthing MJ, Clark ML. Low gluten diet in the treatment of adult celiac disease: effect on jejunal morphology and serum anti-gluten antibodies. Gut 1988;29:1564–8.

28. Hischenhuber C, Crevel R, Jarry B, et al. Review article: safe amounts of gluten for patients with wheat allergy or celiac disease. Aliment Pharmacol Ther 2006;23:559–75.

29. Case S. Gluten-free diet. A comprehensive resource guide. 2nd ed. Regina, Canada: Case Nutrition Consulting, 2006.

30. Collin P, Thorell L, Kaukinen K, Mäki M. The safe threshold for gluten contamination in gluten-free products. Can trace amounts be accepted in the treatment of celiac disease? Aliment Pharmacol Ther 2004;19:1277–83.