Toxicity of gluten traces in patients on treatment for celiac disease: a prospective, double-blind, placebo-controlled gluten trial to establish safe gluten threshold

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Running head: toxicity of gluten traces in celiac disease
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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 for treated patients.

**Objective:** To establish the safety threshold of prolonged exposure to gluten traces. **Design:** This was a multicenter, double-blind, placebo-controlled, randomised trial. Patients were 49 adults with biopsy-proven CD and on treatment with the gluten-free diet (GFD) for at least 2 years. The background daily gluten intake was maintained below 5 mg. After baseline evaluation (T₀), patients were assigned to ingest daily and for 90 days a capsule containing 0 mg, 10 mg or 50 mg of gluten. Clinical, serological and small intestine histology evaluations were performed at T₀ and after the gluten micro-challenge (T₁). **Results:** at T₀ the small intestinal mucosa showed a significantly lower median villous height/crypt depth (Vh/Cd) (2.20; 95% CI 2.11, 2.89) and higher intraepithelial lymphocyte (IEL) count (27; 23, 34) in celiacs than in 20 non-celiac controls (Vh/Cd 2.87; 2.50, 3.09 and IELs 22; 18, 24, respectively). One patient (challenged with 10 mg of gluten) developed a clinical relapse. At T₁ the Vh/Cd % change was 9 (3, 15) in the placebo (n=13), -1 (-18, 68) in the 10 mg (n=13) and -20 (-22, -13) in the 50 mg (n=13) groups. No significant differences in the IEL count were found among the 3 groups. **Conclusions:** The ingestion of contaminating gluten should be kept lower than 50 mg/day in the treatment of CD.

**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 individuals. This is a life-long disorder affecting 0.5 - 1% of the general population worldwide. Treatment of CD is based on the complete avoidance of gluten proteins from the diet, the so-called gluten-free diet (GFD). In the long term (1-2 years) the GFD is associated with clinical, serological and histological remission (1). However it is almost impossible to maintain a “zero gluten level” diet, as 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 or ice creams. 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 due to the presence of wheat starch as a major ingredient.

The potential toxicity of gluten traces is still unclear. We previously showed that, in treated celiac patients, the 4-week ingestion of 100 to 500 mg of gliadin per day (roughly equivalent to 200 – 1,000 mg of 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, as the daily ingestion of contaminating gluten in apparently well-treated CD patients is most likely to be in the range between 5 and 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 NIH Consensus Conference position on CD projected as many as 3 million people being affected by CD in the U.S.A. These findings, together with the recently approved Food Allergen Labelling 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. In order to address the aforementioned issues we undertook a prospective, double-blind, placebo-controlled multi-centre 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 here the final results of this study.

**Study-design, subjects and methods**

**Study-design.** This was a prospective, multi-centre, placebo-controlled, double-blind, randomised trial performed between the years 2001 and 2004. The patients were adults with biopsy-proven CD on a GFD for at least 2 years and in apparent good health. Exclusion criteria were: (a) patients younger than 18 years old; (b) poor compliance to the GFD; (c) abnormal results at the T₀ baseline evaluation, i.e. positivity of anti-tissue transglutaminase (tTG) antibodies and/or definitely abnormal small intestinal biopsy (villous height/crypt depth (Vh/Cd) lower than 1.5); (d) associated conditions, such as selective IgA deficiency or other autoimmune diseases. Patients that qualified for the study were interviewed and gave their informed consent. Controls (only for comparison of the morphometry values at baseline) were adult subjects showing a negative result of the serological CD markers and the urea Breath Test for Helicobacter pylori, and undergoing upper endoscopy for diagnostic purposes.

The patients qualifying for the trial underwent a screening visit and a dietary interview (T₁₋₁). They were asked to maintain a strict GFD during the study-period, avoiding any possible source of gluten contamination (such as consuming meals at restaurants). The only cereal-based food they were allowed to eat were the special GFD products on the market in Italy, which Italian law establishes as having a gluten contamination of less than 20 parts per million (20 ppm = 20 mg per Kg of product). After one month they returned for a baseline evaluation (T₀). This involved (a) clinical examination, (b) a dietary interview, (c) blood testing for serum anti-tTG antibody and antigliadin antibody (AGA) determination and (d) an endoscopy and small intestinal biopsy. While still adhering to a strict GFD, patients were then randomised (by the coordinating center) to ingest daily and for 90 days a capsule containing either 10 mg of purified gluten, or 50 mg of purified gluten or 50 mg of corn starch as a placebo (double-blind micro-challenge). After completing the 3-month micro-challenge (T₁) the patients repeated the same clinical, serological and histological tests as in T₀. If any patient presented symptoms suggestive of CD relapse during the micro-challenge, the protocol was stopped and the patient was asked to perform the T₁ evaluation before dropping out of the study. From T₁₋₁ to T₁ adherence to both the GFD and the study-protocol, together with the clinical progress, were checked on a weekly basis 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, UK). We used whole gluten rather than single gluten fractions, as it has been shown that not only gliadins but also glutenins (the other major component of gluten) contain toxic epitopes (9). Gelatine capsules quickly dissolving into the stomach were prepared by the pharmacy of the coordinating centre. On a dry weight basis, the capsules contained 10 mg of raw gluten, 50 mg of raw gluten or 50 mg of corn starch (placebo). All laboratory tests and analysis 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 ELISA methods (Alfa-Gliatest and h-TTG, Eurospital Trieste, Italy).

Small bowel biopsies (at least 4 specimens for 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 of 5 μm were stained with haematoxylin and eosin and immunostained using anti-human CD3 antibody (DAKO, Glostrup, Denmark) to enhance diagnostic accuracy in counting intraepithelial lymphocytes (IELs). Only well-oriented sections were examined, and when necessary the samples were dissected again until they were of good quality. The specimens were examined in batches by two pathologists with long standing experience in morphometric analysis (I.B., A.M.), who were blinded as to the clinical data. The morphometric analysis of the sections was performed on at least ten well-oriented villi, arranged like fingers, and ten well-oriented crypts,
arranged perpendicular to the muscolaris mucosae, by a computerised image analyser IBAS-AT Kontron (Munich, Germany). The following parameters were evaluated: villous height, crypt depth, Vh/Cd, and IEL count. The number of IELs per 100 enterocytes were calculated computing the mean value out of at least 1000 enterocytes (2). The IEL count was performed on 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. Consumption of gluten-free flour was measured in a different sample of 46 adults with CD (30 women, 16 men; mean age 36 years, range 19-63) on long-term treatment with the GFD (more than 2 years). These subjects were asked to report the type and the amount of all the special gluten-free products consumed during 30 consecutive days in a diary. A random sample of 42 gluten-free products consumed by these subjects was collected and analysed for gluten contamination using an ELISA method (Ridascreen Gliadin, R-Biopharm AG, Germany) with a sensitivity limit of 3 ppm of gluten.

Statistical analysis. Sample size was determined to detect a mean effect size of 0.3 for changes in morphometric variables before and after the micro-challenge, which was expressed as a percentage of baseline values among the three groups of patients, using a significance level of 5%, a power of 85%, and one-way analysis of variance for data analysis. The required number of patients was 39 subjects (13 for each group). Considering a 20% rate of drop-out, 49 subjects were recruited. The normality of distribution of the examined variables was assessed using the Shapiro-Wilk test. Non-parametric methods were used for the statistical analysis of data, since the morphometric variables were not normally distributed. A non-parametric analysis of variance (Kruskal-Wallis test) was performed to evaluate the treatment groups (placebo, 10 mg/day and 50 mg/day) at the baseline. The Wilcoxon test was used to compare pooled CD patients with controls at baseline. The correlation between Vh/Cd and IELs was analysed by Spearman’s coefficient. Changes in morphometric variables before and after the micro-challenge were expressed as percentages of baseline values. The comparisons between the 3 groups were performed by the Kruskal-Wallis test. Since 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%.

Results were expressed as medians and 95% confidence interval (95% CI). A 5% probability was used to assess the statistical significance.

Results
 Forty-nine subjects were enrolled in the micro-challenge study, 37 women and 12 men (median age 30.5 years, range 19.8 – 55.4; median BMI 21.9, range 16.8 – 30.1). After T₀, 7 out of the 49 patients had to be excluded because of abnormal small intestinal histology (4), development of thyroid carcinoma (1), gastric polyposis at baseline gastro-duodenoscopy (1), or because the subject refused randomization (1). Three other cases did not complete the micro-challenge because of change of residence (1 challenged with 50 mg), poor adherence to the protocol (1 challenged with 10 mg), and development of symptoms (1 challenged with 10 mg). Thirty-nine patients completed the study protocol. They were 30 women and 9 men, median age = 30.6 (range 19.8 – 55.4 years), median years on GFD = 10.1 (range 2.1 – 28.0). Of these 39 subjects, 13 were challenged with placebo, 13 with 10 mg and 13 with 50 mg of daily gluten.

Controls were 20 subjects (13 females and 7 males) with a median age of 34.5 (range 24.0 – 60.0) and a final diagnosis of functional dyspepsia (13 cases) or gastro-esophageal reflux (7 cases).

Clinical and serological evaluation. One patient challenged with 10 mg of daily gluten showed typical signs of relapse (vomiting, diarrhoea, abdominal distension) after 6-8 weeks of micro-challenge but refused to repeat the T₁ evaluation. The comparison between the baseline and the post-challenge findings in the 39 patients completing the micro-challenge did not show any significant changes in the clinical outcome among the three groups. The results of the serological CD markers are shown in figures 1A and 1B. In all patients the IgA anti-tTG and IgG AGA
determinations were in the normal range, both at baseline and after the micro-challenge. No significant difference was detected between the three 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 challenged with 50mg of gluten in comparison with the placebo ($P = 0.04$).

**Small bowel biopsy results.** Table 1 shows the results of the small intestinal morphometry in CD patients and controls at baseline. The Vh/Cd ratio was significantly lower in CD patients than in controls and the IEL count was significantly higher in CD patients than in controls. In the pooled CD group (n=39) the correlation between the Vh/Cd and the IEL count was weak and not statistically significant either at baseline ($r = -0.31; 95\%\ CI -0.56, 0.02; P = 0.06$) or after the micro-challenge ($r = -0.29; 95\%\ CI -0.55, 0.03; P = 0.07$).

The results of the gluten micro-challenge are shown in figure 2 and table 2. No significant difference of the morphometry indexes was found at baseline between groups. After the micro-challenge, the Vh/Cd index improved in 11/13 subjects (85\%; 95\% CI 55, 98) in the placebo group, as compared to 6/13 (46\%; 95\% CI 19, 75) in the 10 mg group and only 2/13 (15\%; 95\% CI 2, 45) in the 50 mg group (% of improved biopsies significantly lower in the 50 mg group than the placebo group). The median percentage variation between $T_1$ and baseline ($\Delta\%$) of the Vh/Cd in the placebo group, but not in the 10 mg and the 50 mg groups, showed a significant improvement. On the other hand, at $T_1$ the Vh/Cd showed a significant decrease in the group challenged with 50 mg of gluten per day. The Kruskal-Wallis test showed a significant difference for $\Delta\%$ Vh/Cd between the placebo and the 50mg group ($P = 0.029$). An increase in IELs was observed in only 5/13 (38\%; 95\% CI 14, 68) subjects in the placebo group as compared to 6/13 (46\%; 95\% CI 19, 75) in the 10 mg group and 8/13 (61\%; 95\% CI 32 – 86) in the 50 mg group. No significant differences in the $\Delta\%$ IEL counts were found among the 3 groups.

**Analysis of background gluten intake.** The daily consumption (mean ± s.d.) of commercially available gluten-free products was 332 ± 98 g (range 177 - 574) in 46 adults on treatment for CD. The median gluten content of the 42 gluten-free products analysed was 5.2 parts per million (ppm = mg/Kg) (range 0 – 50). Using these figures, we estimated that the background gluten intake of the GFD performed by our patients during the micro-challenge study was lower than 5 mg per day.

**Discussion**

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

Despite the restricted criteria adopted in this study, the baseline duodenal biopsy showed evidence of histological damage (decreased median Vh/Cd and increased median IEL count) in adult CD patients on long-term dietary treatment. Furthermore, 4 out of 49 subjects had to be excluded from the protocol because a severe enteropathy (obscuring the possible effects of the gluten micro-challenge) 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 the GFD, despite full resolution of their symptoms (13-15). This finding has been 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, since we noticed a significant improvement in the intestinal architecture (Vh/Cd ratio) in the placebo group after 3
months, 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 serological markers are not sensitive enough to detect the residual enteropathy that can be found in apparently healthy CD patients on treatment with the GFD (17-18).

The gluten micro-challenge disclosed a large inter-patient variability of the sensitivity to gluten traces. Some CD patients showed a clear-cut worsening of the small intestinal architecture after ingesting only 10 mg of daily gluten while others had an apparent improvement of the mucosal histology after the 3-month challenge with 50 mg of daily gluten (figure 2). Furthermore, one patient challenged with 10 mg of daily gluten experienced clinical symptoms after a few weeks, while none of the 13 subjects receiving 50 mg had clinical evidence of relapse. Despite this wide individual variability, which must be taken into account in implementing a threshold that is safe for all patients, we showed that 50 mg of daily gluten, if introduced for at least three months, were sufficient to cause a significant decrease of the Vh/Cd in treated celiacs. This is an important new finding, that must however be interpreted with caution, for several reasons. Due to the limited number of patients, we were not able to reach firm conclusions about the potential toxicity of 10 mg of daily gluten, which remained a “grey” area. For ethical reasons we had to limit the duration of the micro-challenge to 3 months, 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).

So far the effects of a low gluten intake in CD patients has been investigated in a limited number of studies. Ciclitira et al 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 moderate change and 1 g extensive damage to small intestinal morphology (22). The same group also reported that the ingestion of 2.4 – 4.8 mg per day of gluten caused no change in the jejunal biopsy morphometry of treated celiacs after either 1 or 6 weeks (3, 23). Eijderhamn et al showed that a daily intake of 4-14 mg gliadin did not affect the morphology of the small bowel mucosa in celiacs on long-term treatment with the GFD (4). Recent Finnish studies indicate that an intake of 20-36 mg of daily gluten has no detectable effect on the mucosal histology (5-6). We previously showed that a 4-week challenge with 100 mg of gliadin per day caused deterioration of the small intestinal architecture and that the histological changes were more pronounced in patients challenged with 500 mg of gliadin per day (2). Finally a higher gluten intake (1 to 5 g of daily gluten), still lower than the normal gluten intake for the non-celiac population in Western countries (10-20 g per day) (24), caused relapse of disease at a clinical, laboratory and histological level, both in children and in adults (25-27). By combining the evidence from the current study and the quoted literature, it appears that 50 mg of daily gluten 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 has recently been reviewed extensively (28-29). In Northern European countries up to 200 ppm of gluten is permitted in food for celiacs, in order to use wheat starch as ingredient. Conversely, a more prudent value of 20 ppm has been adopted in North America and in Southern Europe countries. Based on their clinical and analytical data, Finnish experts recently advocated the “intermediate” limit of 100 ppm (30). The decision on the threshold 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, as the harmful gluten intake of 50 mg could be reached even with a moderate consumption (250 g per day or more) of nominally gluten-free products. A 100 ppm threshold, by allowing up to 10 mg of gluten out of 100 g of product, is also probably not suitable for generalized use, especially in
countries (like Italy) where consumption of wheat substitutes is occasionally as high as 500 g per day (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, therefore allowing a safety margin for the variable gluten sensitivity and dietary habits of patients.

In conclusion, this study confirms that an abnormal small bowel morphology persists in a significant proportion of CD patients treated with the GFD, most likely due to persistent ingestion of gluten traces. The protracted intake of 50 mg of gluten per day produces a significant damage of the architecture of the small intestine in patients on treatment for CD. However the sensitivity to gluten traces shows a large inter-patient variability, a feature that should be accounted for in the implementation of a safe gluten threshold. These findings should be confirmed by further studies on larger samples of CD patients. Finally, the relationship between the intestinal damage induced by gluten traces and the long-term complications of CD remains to be elucidated.

Acknowledgements

This study was primarily conceived and designed by C.C. and A.F., but all the co-authors contributed to the implementation of the study-design. Data were collected by E.F., G.I., C.D., R.F., F.B., U.V., S.A., A.P., I.D., G. P., A.M., I.B. and analyzed by E.F., R.G., and F.C.. The interpretation of the results and the writing of the manuscript were primarily performed by C.C., E.F. and A.F. with the contribution of all the other co-authors. The statistical analysis was performed by R.G. and F.C.

At the time of this study C.C. was a member of the scientific advisory board of the Italian Celiac Society (AIC, a no-profit organization of patients with CD). None of the authors had a financial or personal conflict of interest related to the funding of or the outcome of this research.

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References


Tab. 1. Intestinal morphometric evaluation (expressed as median and 95 % CI) in 20 controls and in 39 treated celiacs investigated before starting the gluten micro-challenge.  

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 20)</th>
<th>CD patients at baseline (n = 39)</th>
<th>P</th>
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<tbody>
<tr>
<td>Villous Height (µm)</td>
<td>372.7 (339.5, 385.4)</td>
<td>354.3 (318.0, 380.5)</td>
<td>0.130</td>
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<tr>
<td>Crypt depth (µm)</td>
<td>135.1 (124.8, 149.7)</td>
<td>150.9 (134.9,160.6)</td>
<td>0.077</td>
</tr>
<tr>
<td>Vh/Cd</td>
<td>2.9 (2.5, 3.1)</td>
<td>2.2 (2.1, 2.9)²</td>
<td>0.019</td>
</tr>
<tr>
<td>IEL count (x 100 enterocytes)</td>
<td>22 (18, 24)</td>
<td>27 (23, 34)²</td>
<td>0.002</td>
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</tbody>
</table>

¹ CD: celiac disease; Vh/Cd: villous height/crypt depth ratio; IEL: intra epithelial lymphocyte 
² significantly different from controls (Wilcoxon test).
Tab. 2. Results of the 3-month gluten micro-challenge in patients with celiac disease (n=39). The intestinal morphometric indexes\(^1\) are expressed as medians and 95% CI. Differences between treatment groups were analysed by the Fisher’ exact test\(^2\) and the Kruskal-Wallis\(^3\) test. Marsh-Oberhuber grading of the small intestinal damage is: 0 (normal); 1 = infiltrative lesion (increased IEL count); 2: hyperplasic lesion (increased IEL count and increased crypt depth).

<table>
<thead>
<tr>
<th>MARSH-OBERHUBER GRADING OF SMALL BOWEL HISTOLOGY</th>
<th>Placebo (n=13)</th>
<th>10 mg gluten /day (n=13)</th>
<th>50 mg gluten/day (n=13)</th>
<th>P</th>
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<tr>
<td>Baseline</td>
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<tr>
<td>0</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>0.263(^2)</td>
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<td>1</td>
<td>4</td>
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<td>5</td>
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<tr>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td></td>
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<tr>
<td>After micro-challenge</td>
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<td></td>
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<tr>
<td>0</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>0.433(^2)</td>
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<tr>
<td>1</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td></td>
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| INTESTINAL MORPHOMETRY AT BASELINE            |              |                |                |     |
| Villous Height (µm)                          | 354 (315, 437) | 318 (306, 366) | 380 (266, 396) | 0.431\(^3\) |
| Crypt Depth (µm)                             | 150 (134, 165) | 161 (124, 187) | 131 (110, 159) | 0.197\(^3\) |
| Vh/Cd                                        | 2.2 (2.1, 3.0) | 2.0 (1.8, 3.4) | 2.4 (2.2, 3.2) | 0.273\(^3\) |
| IEL count (x 100 enterocytes)                | 26 (23, 45)   | 29 (23, 46)    | 27 (17, 48)    | 0.968\(^3\) |

| % MORPHOMETRY CHANGES AFTER THE MICRO-CHALLENGE |              |                |                |     |
| Villous Height                               | 5 (-5, 13)   | 9 (-9, 19)     | -17 (-30, 23)  | 0.198\(^3\) |
| Crypt depth                                  | -2 (-16, 9)  | 3 (-28, 21)    | 3 (-17, 24)    | 0.693\(^3\) |
| Vh/Cd                                        | 9 (3, 15)    | -1 (-18, 68)   | -20 (-22, -13) | 0.029\(^3\) |
| IEL count                                    | -4 (-22, 42) | 0 (-14, 47)    | +12 (-8, 93)   | 0.514\(^3\) |

\(^1\) Vh/Cd: villous height/crypt depth ratio; IEL: intra epithelial lymphocyte
\(^2\) Fisher’ exact test
\(^3\) Kruskal-Wallis test
\(^4\) 50 mg significantly different from placebo (P < 0.05)

Legends

Fig. 1. Median and interquartile levels of serum IgA class anti-tissue transglutaminase (tTG) (A) and IgG class anti-gliadin (AGA) antibodies (B) in the study-group (placebo n= 13; 10 mg of daily gluten, n=13, 50 mg of daily gluten, n= 13). No significant difference at baseline and no significant
increase after the micro-challenge were detected (Kruskal-Wallis test). Open bars: pre-challenge; Dashed bars: post-challenge. Dashed line: normal cut-off; A.U. = arbitrary units.

Fig. 2. Individual results of the small intestinal morphometric evaluation before and after the 3-months gluten micro-challenge in the placebo (n=13), 10 mg gluten/day (n=13) and 50 mg gluten/day (n=13) groups. Vh/Cd: villous height/crypt depth ratio; IEL: intra epithelial lymphocyte; MC: micro-challenge.
Figure 1

A

B

Figure 1
Figure 2