4th of October 2012

Dear Editor,

The authors would like to thank the reviewers for their specific and helpful comments.
Please find enclosed the edited manuscript in Word format (file name wjg-2012-032881-review .doc)

Title: Consumption of gluten with gluten-degrading enzyme by celiac patients: A pilot-study.

Authors: G.J. Tack, J.M.W. van de Water, M.J. Bruins, E.M.C. Kooy-Winkelaar, J. van Bergen, P. Bonnet, A.C.E. Vreugdenhil, I. Korponay-Szabo, L. Edens, B.M.E. von Blomberg, M.W.J. Schreurs[4], C.J.J. Mulder, F. Koning

Manuscript No: wjg-2012-032881

The manuscript has been improved according to the suggestions of reviewers:

1. Format has been updated
   Grammar and typographic errors in the manuscript were corrected.
   References and typesetting were corrected.

Revision has been made according to the suggestions of the reviewer:
2. The abstract has been revised as asked by reviewer 1:
"AIM: The fungus derived enzyme *Aspergillus niger* prolyl endoprotease (AN-PEP) effectively degrades gluten *in vitro*. This study assesses the safety and efficacy of AN-PEP to mitigate the immunogenic effects of gluten in celiac patients."

3. The first reviewer is surprised about the small number of patients.
We used the following sample size calculation: " For a detectable difference of 1.3*σ between the treatment groups given a power of ß = 0.80 and an α = 0.05 (one sided) the sample size should be 2*7 = 14."

4. The second reviewer was wondering why we did chose Marsh grade 0 and I, as these Marsh grades are not indicative for CD.
We do agree with the reviewer that Marsh 0 and I are not in accordance with the diagnoses of CD. There might be some misunderstanding. Only patients who were diagnosed with a florid CD (at least Marsh III) in the past were included, yet at time of inclusion they all had a Marsh 0 or I upon a GFD indicating a proven clinical and histological response upon o GFD. A deterioration caused by gluten exposure can only be found with Marsh score 0 or I at inclusion. We did expect deterioration of the Marsh score after restarting gluten (with placebo)

5. In answer to the reviewers' question about the sufficiency of the 2 week washout period:
As described in the discussion the time to serological and mucosal relapse and recovery after gluten re-introduction and elimination, can be highly variable among adult CD patients from several weeks up to many years. In retrospect a two week wash-out might be too short and a longer gluten challenge might be needed. However, the data available are not consistent. We added the consideration to include a longer wash-out period in a future study to the discussion.

Sincerely yours,
On behalf of the authors,

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30th of September 2012

Dear Editor,

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Please find enclosed the edited manuscript in Word format (file name wjg-2012-032881-review .doc)

Title: Consumption of gluten with gluten-degrading enzyme by celiac patients: A pilot-study.

Authors: G.J. Tack, J.M.W. van de Water, M.J. Bruins, E.M.C. Kooy-Winkelaar, J. van Bergen, P. Bonnet, A.C.E. Vreugdenhil, I. Korponay-Szabo, L. Edens, B.M.E. von Blomberg, M.W.J. Schreurs[4], C.J.J. Mulder, F. Koning

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The manuscript has been improved according to the suggestions of reviewers:
1. Format has been updated
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Comment reviewer 1
This is an interesting and well done study regarding the treatment of celiac patients with an enzymatic approach. Even if the primary endpoint was not met, this study is of interest and warrant support for such an approach.
My only question is the small number of patient.
Minor: in the abstract, first line, define AN-PEP instead of in the second line.

- According to this comment, revision has been made to the abstract.
"AIM*:* The fungus derived enzyme *Aspergillus niger* prolyl endoprotease (AN-PEP) effectively degrades gluten *in vitro*. This study assess the safety and efficacy of AN-PEP to mitigate the immunogenic effects of gluten in celiac patients."
- The first reviewer is surprised about the small number of patients.
We used the following sample size calculation: " For a detectable difference of 1.3*σ between the treatment groups given a power of ß = 0.80 and an α = 0.05 (one sided) the sample size should be 2*7 = 14."

Comment reviewer 2
The aim of the present study was to examined the the safety and efficacy of Aspergillus niger prolyl endoprotease (AN-PEP) to reduce the clinical response to gluten in patients with Coeliac Disease (CD). The safety study showed that AN-PEP was safe and well tolerated by patients with CD. However, the efficacy study showed a lack of clinical response due to an inappropriate gluten challenge mainly in the placebo group. The authors suggested that a longer gluten challenge may be necessary to induce a clinical response to gluten. It is perhaps that the cohort selected Marsh score 0 or 1 may not be be classified as CD. Why did the authors chose Marsh score of 0 and 1? A marsh score of 0 is not indicative of CD.

Overall this is a novel and original study and has provided some good evidence that AN-PEP may have some potential therapeutic effects in CD. It is worth publishing. The manuscript was well written with great detail. However, further clarification is needed;
(1) Was a washout of 2 week sufficient?
(2) Why chose Marsh score 0 and 1 as CD patients? Marsh score 0 have normal histology. This may be why you are see a lack of clinical response with the gluten challenge.

- In answer to the reviewers' comments about the wash-out period:
As described in the discussion the time to serological and mucosal relapse and recovery after gluten re-introduction and elimination, respectively, can be highly variable among adult CD patients from several weeks up to many years. In retrospect two weeks wash-out might be too short and a longer gluten challenge might be needed. However, the data available are not consistent.

We added the consideration to include a longer wash-out period in a future study to the discussion.

- In answer to the reviewers' comments about the chosen Marsh scores 0 and 1.
We have selected patients who had high Marsh scores at time of diagnosis and who had well-controlled coeliac disease at time of inclusion because deterioration caused by gluten can only be found with Marsh score 0 or I at inclusion.

3. Grammar and typographic errors in the manuscript were corrected.
4. References and typesetting were corrected.

Sincerely yours,
On behalf of the authors,

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